Calcium Intake of Chinese Pre-school Children in Hong Kong

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List of Abbreviations

Average daily calcium intake over the first five years of life	AvCa
Average daily energy intake over the first five years of life	AvKcal
Average daily protein intake over the first five years of life	AvProt
Bone Mineral Content	BMC
Bone Mineral Density	BMD
Bone Width	BW
Height	HT
Kiloelectronvolt	KeV
Parathyroid Hormone	PTH
Recommended Dietary Allowances	RDA
Sun Yat Sen University of Medical Science	SYSUMS
Weight	WT
1,25-dihydroxycholecalciferol	$1,25(OH)_2D_3$

Summary

Insufficient accumulation of bone mass in children has been speculated as a predisposing factor to fractures later in life. Hence, there is a growing concern for giving adequate calcium to children in order to achieve peak bone mass in adulthood. The aim of this study was to find out whether a higher calcium intake would lead to a higher bone mass of children in Hong Kong.

128 Hong Kong Chinese children (67 boys, 61 girls) who had been recruited for an on-going longitudinal study on growth and nutrition participated in the study. Calcium intake from birth to five years and bone mineral content (BMC) at five years were determined. Dietary intake was evaluated by a combination of three methods: dietary history record, food frequency record and 24-hour recall. BMC was determined by single photon absorptiometry, using Norland model 2780 Bone Densitometer, at the distal one-third site between the styloid process and the tip of the olecranon of the right radius.

At five years, the mean daily calcium intake was 546 mg/day (SD=325). Milk was the chief source of calcium (43.5%). 90% of all the studied children have been taking milk regularly from birth to five years of age. The mean (SD) BMC and bone width of these children were 0.317 (0.042) g/cm and 0.756 (0.074) cm respectively. The BMC

shows significant correlation with weight, height and bone width but not with calcium, energy and protein intakes.

When dietary calcium intake of the previous five years was taken into account, the cumulative calcium intake over the first five years is significantly correlated with BMC ($r=0.235$, $p=0.0133$). The correlation remains significant even after adjusting for sex, body size, bone width and energy intake ($r=0.248$, $p=0.0107$). When the children were divided into low, medium and high calcium intake groups, the BMC of the medium and high calcium intake groups are significantly higher than that of the low calcium intake group ($p < 0.05$). It is concluded that the cumulative intake of calcium in the past years of life is significantly correlated with BMC in children at five years. Moreover, the calcium intake particularly in the second year of life is highly correlated with BMC at five ($r=0.240$, $p=0.02$). In addition, the body size and bone width are also important independent determinants of BMC.

Although bone mineral density (BMD), a ratio of BMC to bone width, was found to be significantly correlated with calcium intake at five years ($r=0.193$, $p=0.029$) and calcium intake over the first five years of life ($r=0.2017$, $p=0.035$), the use of BMD as a parameter to express bone mass is still an unproved conceptual adjunct.

Children in Mainland China are known to have low calcium intake and this was confirmed in a pilot study of 16 children at age five living in Jiangmen of Guangdong Province in China. Calcium intake of Jiangmen children was 244 mg/day (SD=46) which was only half of those in Hong Kong. The BMC of 115 Jiangmen children (63 boys, 52 girls) was also measured. The BMC of Hong Kong children was significantly higher than their counterparts in Jiangmen even after adjusting for body size. Using multiple regression analysis, the difference in BMC between Hong Kong and Jiangmen could not be fully explained by the difference in calcium intakes at five years. The different levels of cumulative calcium intakes from birth to five years or the different degrees of physical activity would possibly explain the difference.

A continual consumption of milk at least up to the second year of life in children of Mainland China appears to be beneficial for attaining a higher bone mineral content.

To conclude, the calcium intake of Hong Kong Chinese pre-school children was found to be comparable with recommended dietary allowances. 90% of children were taking milk regularly and these children were healthy and growing normally. Therefore, as a group the calcium intake of Hong Kong Chinese pre-school children is adequate. Children with higher calcium intake in the past years is found to have higher BMC. This study indicates that calcium intake on a long term basis has an important effect on BMC in growing children. To study the relation between calcium intake and BMC by cross-sectional study may not be valid to reveal the actual relationship. Further study to investigate the beneficial effect of higher BMC at five that might influence peak bone mass in mature years is warranted.

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Chapter 1

Introduction

1.1 Calcium Nutrition and Bone Health

From a physiological point of view, more than 99% of the total body calcium is stored in the skeleton, it would be plausible to speculate that a low intake of the element might prejudice the quality and quantity of bone, particularly in fast growing children. In theory, an inadequate intake of calcium might also impair vital functions relating to calcium, namely membrane permeability, nerve conduction, muscle contraction and blood coagulation, etc.

It has been suggested that an insufficient accumulation of bone mass in childhood and early adulthood might predispose to fractures later in life when age-related bone loss commences (Riggs & Melton 1986; Marcus 1987; National Research Council 1989). Hence, there is a growing concern for achieving peak bone mass during the years of bone mineralization. And peak bone mass appears to relate to calcium intake during the period of bone growth (Matkovic, Kostial, Simonovic, Buzina, Brodarec & Nordin 1979).

Most of the bone mineral attains by 20 years of age and consolidation of bone mineral continues during the third decade (Dequeker 1988; National Research Council 1989). Bone mass declines gradually after reaching the peak. In women the rate of bone loss accelerates as soon as menopause begins. While in men the loss progresses slower and occurs a decade or more later in life. This results in diminishing bone strength and increasing risk of fractures (Dequeker 1988). The condition is called osteoporosis.

In view of the rising incidence of age-related fractures in many parts of the world including Hong Kong (Lewis 1981; National Institute of Health 1984; Lau & Donnan 1987; Lau 1988; Lau & Donnan 1990), numerous research has been conducted to search for strategies in preventing the disease.

One of the widely supported hypotheses in reducing the risk of osteoporotic related fractures, though not yet proven, is to ensure optimal calcium intake throughout childhood and right into the late twenties in order to attain peak bone mass (Matkovic et al. 1979; Sandler, Slemenda, LaPorte, Cauley, Schramm, Barresi & Kriska 1985; National Research Council 1989). The positive effect of calcium intake on bone mass has been recognised in several retrospective studies. A study in postmenopausal women (Sandler et al. 1985) using retrospective dietary assessment method reported that women with higher milk consumption in childhood and adolescence had significantly higher bone mass than women who reported to be drinking milk less frequently. This study suggested that a higher calcium intake in childhood and adolescence might be associated with higher bone mass later in life. Picard, Ste-Marie, Coutu, Carrier, Chartrand, Lepage, Fugere and D'Amour (1988) studied premenopausal bone mineral content of 183 Caucasian women in relation to calcium intake during early adulthood (since age 20). The results indicated that a higher calcium intake during early adulthood was related to higher axial and appendicular bone mass in premenopausal women. Furthermore, the authors suggested that calcium intake in early adulthood would affect peak bone mass in premenopausal women. A recent study (Halioua & Anderson 1989) looking into the life time calcium intake and life time physical activity of 181 premenopausal Caucasian women found that the life time calcium intake was a significant predictor of bone mass of the axial bones and the distal forearm. The authors emphasized the importance of calcium intake during adolescence and early 20's when consolidation of the skeleton was taking place. Kelly, Pocock, Sambrook and Eisman (1990) studied the effect of current calcium intake that might influence bone mineral density of axial and appendicular bone of adult men. They showed that dietary calcium intake was an important independent predictor of bone mineral density of axial bones. A higher calcium intake was also associated with greater bone mineral density at the distal radius. Dietary calcium intake explained 24% and 42% of the variance of bone mineral density at the lumbar spine and the femoral neck respectively.

To conclude, these studies demonstrated that an adequate calcium intake in childhood, adolescence and early adulthood had an important role in enhancing peak adult bone mass.

Hence, in the tenth edition of the American

Recommended Dietary Allowances (National Research Council 1989), the daily calcium allowance (1,200 mg/day) recommended for the age between 11 to 18 has been extended to age 24. Yet there has been no prospective controlled study to support the view that higher bone mass accumulated in childhood would ensure optimal peak adult bone mass or protect against fractures in later life.

1.2 Calcium Requirements in Children

From infancy to adulthood, the body calcium store increases from about 30g to about 850 - 1,400g (American Academy of Pediatrics, 1978). In order to accumulate sufficient amount of calcium at maturity, it was estimated that during the first six months of life, the daily calcium accretions in the skeleton is about 150 - 200mg/day, less influx (75 to 100mg/day) in mid-childhood years, and up to 400mg/day during the growth spurt in adolescence (American Academy of Pediatrics, 1987).

From infancy to late adolescence, bone formation exceeds bone degradation (Guyton, 1981; Schaafsma, 1988), the formation of bone in children, requires a positive calcium balance (Nordin & Marshall, 1988). The requirement of calcium in children may be defined as the amount of calcium required to maintain a positive balance; which in turn is determined by the amount of calcium absorbed from the intestine, the calcium required for bone formation, its requirement for maintaining the extracellular pool and the obligatory loss of calcium from skin and kidney (Fraser, 1988b).

In adults the body is found to be capable of adapting to low calcium intake. Under this circumstances, the rate of calcium absorption is enhanced in gut whereas urinary loss of calcium is reduced (Hegsted, Moscoso & Collazos 1952; Malm 1958; Kanis & Passmore 1989). However, the success of adaptation requires an adequate dietary supply of vitamin D (Hegsted et al. 1952; Fraser 1988b; Kanis & Passmore 1989). Nevertheless, adaptation to low calcium intake may take months to complete (Malm 1958). There is no evidence that this adaptation does not occur in children at a low plane of calcium nutrition (Fraser 1988b).

Calcium intakes of children in different cultures vary greatly. In communities where milk is not readily available or is not traditionally consumed, habitual calcium intakes are often below the recommended levels of intakes (FAO/WHO 1962; Nordin & Marshall 1988). When growing children are subject to low calcium intake, the synthesis of vitamin D increases to enhance intestinal calcium absorption and urinary excretion of calcium reduces. However, bone is more resistant to calcium resorption in favour of bone synthesis. (Fraser 1988b; Walker 1972; Schaafsma 1988; Peacock 1988). In addition, Fraser (1988b) suggested that calcium absorption in the colon of children may increase in response to chronic low calcium intake.

In the western world the estimates of calcium requirements in children have been basically extrapolated from balance studies conducted in adults (Nordin & Marshall 1988). These balance studies do not consider the ability of the body in adapting to various levels of calcium intakes. In fact, calcium required to maintain positive balance varies with previous intakes, a high habitual calcium intake requires higher requirement of calcium to maintain positive balance (Council on Foods and Nutrition 1963; McBean & Speckmann 1974).

Because of this adaptive response, calcium requirements cannot be simply determined by estimating positive calcium balance at various levels of intakes over a relative short period of time unless the period of balance study is sufficiently prolonged such that full adaptation can be assumed. Therefore, the estimation of calcium requirements based on sudden decrease in calcium intake is liable to error (Kanis & Passmore 1989).

The recommended dietary allowances (RDA) for calcium in children vary from 400mg/day to 1000mg/day (Nordin & Marshall 1988). These nutrition committees assume that the rate of intestinal calcium absorption is only about 20-35% (Fraser 1988b; Nordin & marshall 1988). Furthermore, they consider that it is important to supply plenty of calcium in early life to achieve peak bone mass at maturity so as to prevent osteoporosis in advanced age. In addition, a higher calcium intake is recommended because of higher animal protein and salt intakes in the western societies (Worthington-Roberts 1981b).

The RDA in the U.S.A. has been traditionally set at two standard deviations above the mean requirements which aims at providing a wide margin of safety above the needs of most healthy people in the country (Guthrie 1989). Consequently, the levels of RDA for calcium in the affluent western societies may be inflated and might not truly indicate the actual mean requirements, particularly in people with low milk intake, less consumption of protein and salt.

FAO/WHO (1962) recommended calcium intakes for children range between 400 to 500 mg/day. The committee remarked that calcium intake above this level is considered to be safe whereas intake below this level might carry some risk of deficiency. Many population groups consume considerably less than this recommended level of intake, especially if milk is not included in diet (Nordin & Marshall 1988).

Children subsist on habitual low calcium diet, growth does not seem to be different from their higher calcium intake counterparts. Unequivocal evidence of calcium deficiency in children with low calcium intake is lacking (Council on Foods and Nutrition 1963; Paterson 1978; Walker 1972; Kanis & Passmore 1989). A study carried out in Surinam found that the thickness of the cortical bone of Javanese school children taking an average of 30 ml milk daily was not significantly different from the counterparts in boarding school consuming an average of 400 ml milk daily (Luyken & Luyken-Koning 1969). Walker (1972) assembled evidence that the dimensions, composition and density of bone from children in communities with habitual low calcium intakes were similar to those with high calcium intakes. In U.S.A., the mean bone density of the black children are higher than that of Caucasians in spite of the fact that intakes of calcium are usually lower in the black children (Garn & Clark 1975).

1.3 Concern for Calcium Intakes in Hong Kong Chinese

1.3.1 In Adults

Traditionally, Milk and milk products are not commonly consumed in the Chinese diet. Few adult Chinese take milk regularly. Thus, the Chinese diet has been characterised as low in calcium (Pun, Chan & Chung 1989), this dietary characteristic is attributable to the traditional eating habits and lactose intolerance in a substantial proportion of the Chinese population (Quak, Raman, Low & Wong 1987; Dahlqvist 1984).

Some Studies have reported that in populations with higher calcium intakes throughout life, their bone mass is higher and their risk of developing fractures is lower in comparison with their low calcium intake counterparts (Matkovic et al 1979; Sandler 1985). The association of higher bone mass attained at matured years with higher calcium intake in childhood and early adulthood is presumed to be protective against age related bone loss in later life (Matkovic et al 1979).

Pun et al. (1989) studied calcium intake in 535

Hong Kong Chinese. They found that calcium intake rapidly declined with age, the average calcium intake was about 500mg/day between 11 and 20 years of age and dropped to around 300mg/day at 70 years of age. In recent years, osteoporotic fractures among the elderly population is an increasing public health problem in Hong Kong (Lau & Donnan 1987; Lau 1988; Pun & Chung 1989). Lau, Donnan, Barker and Cooper (1988) studied the calcium intake of the elderly with hip fractures. It was found that calcium intake of the elderly patients with fractures was significantly lower than the normal controls.

1.3.2 In Children

From early 1960's to late 1970's, studies concerning growth and dietary intakes of Hong Kong infants and young children have painted a gloomy picture on the nutritional status of these children (Field & Baber 1973; Li, Baber Yu & Leung 1982; Li, Baber & Leung 1985). In 1960's, Field and Baber (1973) conducted a longitudinal study of growth in 782 children in Hong Kong. They reported that by nine months of age, most children had abstained from milk drinking. Parents generally perceived that milk was required for the young babies only. Inadequate consumptions of food and nutrients were common in these children.

In 1979 and 1981, Li et al. (1982, 1985) carried out two dietary surveys in Hong Kong Chinese infants and young children between seven months to 24 months of age in the districts of Aberdeen and Kwun Tong respectively. The authors revealed a poor nutritional status among the studied young children whose diets were markedly low in nutrients, including calcium. The reports urged for an improvement in child nutrition in Hong Kong.

Nonetheless, Leung, Lui and Swaminathan (1989), and Leung and Lui (1989) published some interesting findings from their cohort study of growth and nutrition in 150 normal Chinese Children from birth to 18 months in Shatin (a new town in Hong Kong). They noted some remarkable changes in feeding patterns of young children since the 1960s. A majority of the studied children were bottle fed, of whom 74% at day seven, 92% and 97% at two months and six months respectively. These bottle fed children were all fed with fully fortified infant formulae which were continued to be given throughout the weaning period. Therefore, the intake of calcium should be adequate during the weaning period. One-third of the children around four months of age started with fortified rice cereals. By six months, rice congee (rice porridge cooked with water) prepared with meat or fish was introduced to half of the children. The amount of solids and the concentration of the congee were gradually increased until about 18 months when the children could manage to take a mini adult diet.

The energy intake of these Chinese children compared favourably with those of Canadian and Australian counterparts. The authors commented that these children were all well nourished. These remarkable changes might be attributable to the achievements in the economic progress as well as an improvement in general education for the public over the last three decades.

However, these findings were only relevant to young children till 18 months of age. There has been no published data to determine the current nutritional status, particularly the calcium intake of pre-school children from two to five years in Hong Kong.

1.4 Aims of the Study

In view of the significance of calcium intake in early childhood that might influence the maximum bone mass attained in adulthood, the necessity in evaluating the current level of calcium intake and the baseline bone mineral content in Hong Kong Chinese pre-school children is warranted. Furthermore, to examine whether there is any significant relationship between calcium intake and bone mineral content as early as five years of age.

Chapter 2

Calcium Homeostasis

2.1 Calcium in Body Skeleton

The adult body contains about 1.2 kg of calcium, more than 99% of which is present in the skeleton in the form of calcium phosphate, which constitutes most of the hard structure of the skeleton. Bone is constantly undergoing turn over, a continuous process of bone formation and resorption. In childhood and adolescence, the rate of formation of bone mineral predominates over the rate of resorption. During adulthood, both rates are in equilibrium. In later life, resorption predominates over formation, and gradually bone loss proceeds to noticeable levels. Garn (1970) has concluded that adult bone loss is a normal phenomenon in human.

In normal adults, the supply of calcium to the extracellular pool comes from the dietary source. The obligatory endogenous losses of calcium via kidney, intestine, and skin require regular influx of calcium into the extracellular pool. If there is a prolonged insufficiency of calcium intake, either malabsorption of calcium or excessive calcium loss, the bone will be actively involved in maintaining calcium concentration in the extracellular pool. Calcium in bone will be resorbed to maintain the level in the plasma pool because bone is the main repository of body calcium.

However, in growing children, bone plays a less active role in contributing to the extracellular fluid under adverse circumstances since the growth of bone predominates at this stage of life (Schaafsma 1988). Theoretically, a persistent inadequate intake of calcium would retard bone growth but so far there is no evidence to support it (Walker 1972).

2.2 Hormonal Regulation of Calcium Concentration in the Extracellular Fluid Compartment

The plasma calcium concentration is maintained within narrow limits, about 2.25 - 2.6 mmol/l. This is mainly regulated through the integrated effects of three hormones: parathyroid hormone (PTH), calcitonin and metabolically active vitamin D_3.

A fall in the concentration of circulating calcium causes a rise in the secretion of PTH. PTH stimulates the synthesis of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) which is an active metabolite of Vitamin D_3. $1,25(OH)_2D_3$ promotes absorption of calcium from the intestine and increase resorption of calcium from bone. In addition, PTH stimulates reabsorption of calcium from the kidney tubules. Thus, reducing the loss of calcium through the kidney. On the other hand, a rise in serum calcium concentration promotes a decrease in PTH secretion and an increase in calcitonin release. This results in a decrease in production of $1,25(OH)_2D_3$, which leads to reduced intestinal absorption, bone resorption of calcium and calcium reabsorption from renal tubules. Consequently, the plasma calcium concentration is restored to normal level.

2.3 Calcium Absorption

Most dietary calcium exists in complex forms with some food constituents. For example, calcium caseinate in milk and calcium phosphate in vegetables, etc. These complexed calcium is hardly soluble in neutral solutions but are readily soluble below pH 6. (Bo-Linn, Davis, Buddrus, Morawski, Santa Ana & Fordtran 1984). The calcium complexes must be dissociated by digestive enzymes and the released calcium needs to be in a soluble and probably in an ionised form before absorption occurs (Nordin 1968; Schacter, Dowdle, & Schenker 1960).

The presence of gastric acid appears to increase the solubility of calcium complexes in rats (Mahoney, Holbrook & Hendricks 1975). However, Bo-Linn et al.(1984) could not demonstrate this phenomenon to happen in human. Bile salts released in the duodenum appears to increase the in vitro solubility of calcium and hence the absorption of calcium in the intestine (Wills 1973). It seems that calcium is more soluble in duodenum and proximal jejunum (Allen 1982; Bronner 1988).

2.3.1 Calcium Transport Across the Intestine

The intestinal absorption of calcium involves two transport mechanisms: active calcium transport (saturable process) which occurs mainly in the proximal small intestine; and passive calcium transport (non-saturable process) which takes place in the rest of the intestine (Bronner 1987). The calcium transport in the saturable process is mediated by the vitamin D dependent calcium binding protein. Whereas the absorption of calcium via the non-saturable process is not subject to hormonal control (Bronner 1988).

2.3.1.1 Active Calcium Transport

The luminal calcium diffuses into the enterocytes moving down the calcium concentration gradient. The calcium concentration gradient varies between 10 to 100 folds between luminal and serosal fluid depending on the level of calcium intake. In addition, the chemical gradient that exists between cell exterior and interior is a thousand times in difference. Therefore, the entry of calcium into the epithelial cells occurs readily without expenditure of energy (Bonner 1987). In addition, vitamin D assists in enhancing calcium entry by 20 - 30% through the brush border membrane, possibly via a membrane bound calcium binding protein (Bronner, Pansu & Stein 1986).

An intracellular calcium binding protein may be responsible for facilitating calcium transport through the cytosol (Feher 1983). There is about 60 folds of increase in intracellular calcium movement as a result of the facilitation by calcium binding protein [Bronner et al 1986]. Study has shown that there is a linear relationship between the rate of calcium transport and the concentration of binding protein (Pansu Bellaton &

Bronner 1983). In the study of rat, active transport of calcium in the intestinal cells does not occur in vitamin D and calcium binding protein deficient rats (Bronner et al 1986).

Calcium extrudes into the extracellular fluid from the serosal side of the intestinal cells. However, it occurs against both the chemical gradient of calcium/magnesium - ATPase and the electrical gradient (Bronner 1987). Calcium-magnesium ATPase has the capacity to extrude calcium without aid from calcium binding protein (Bronner 1987). Like calcium entry into the brush border membrane, vitamin D may also enhance extrusion of calcium into the extracellular fluid by amplifying the activity of calcium-magnesium ATPase by two to three folds (Bronner et al. 1986).

Calcium binding protein was found to be vitamin D dependent in the process of active calcium transport. The calcium binding protein mediates and enhances intracellular calcium diffusion (Henry & Norman 1984). Moreover, this active process occurs predominately in the duodenum and the proximal jejunum (Behar, Kerstein 1986; Bronner et al 1986).

2.3.1.2 Passive Calcium Transport

This process takes place predominantly in the distal jejunum and the ileum (Pansu et al 1983; Behar & Kerstein 1976). It is believed that the route of entry into the epithelial cells is through the paracellular route (Nellans & Kimberg 1979) but not via the transcellular route as seen in the saturable calcium transport. Modifications of the cellular junctions, namely, the tight junctions and the gap junctions may lead to a marked influx of calcium (Bronner 1987). And the non-saturable route of calcium transport is concentration dependent (Bronner 1987). At low calcium intake, calcium is mainly absorbed by active transport. However, with increasing intake this mechanism is saturated and additional calcium is absorbed by diffusion paracellularly (Nordin & Marshall 1988).

As a result, varying levels of calcium intake would result in two effects that tend to work in opposite directions: at low calcium intake, active transport is up-regulated via the increased expression of vitamin D. When calcium intake is raised, active transport is down-regulated via the diminished expression of vitamin D action. On the other hand, passive transport varies directly with calcium intake. It goes up when calcium intake increases and goes down when calcium intake reduces.

2.4 Effects of Age on Calcium Metabolism

The concentration of plasma ionised calcium varies slightly throughout life and there is no gender difference in plasma ionised calcium (Marshall 1976).

It was reported that calcium absorption in infants is about 70% of the ingested calcium at an intake of 280 mg/day (about 800 ml of milk volume). And the absorption tends to decline from infancy to adulthood, and it declines further in old age (American Academy of Pediatrics 1978). Although calcium intake of a child may be as high as an adult, urinary calcium excretion is lower in children than adults. It is because calcium absorption is more efficient in children than in adults, In growing children calcium is retained for bone development. Hence, little calcium is excreted in urine (Peacock 1988).

Age related decline in intestinal calcium absorption has been shown in both human and in animals (Allen, 1982). This is due to a decline in the active process of calcium absorption in the proximal small intestine. Such observation is also in parallel with a decrease in the synthesis of vitamin D in the elderly who are secondary to age-related declining renal function (Peacock 1988).

2.5 Effects of Nutrients on Calcium Bioavailability

It is known that certain food constituents may affect the bioavailability of calcium. The term Bioavailability is defined as the proportion of the ingested nutrient that can be digested, absorbed, and utilised within the body (Southgate 1989). Certain food constituents, for instance, Vitamin D (Worthington-Robert 1981a), lactose (McBean & Speckmann 1974) and glucose (Zhang 1989), etc., have been found to promote calcium absorption in gut. Whereas other food components, for example, phytic acid (McBean & Speckmann 1974, Graft &

Eaton 1984) and oxalic acid (McBean & Speckmann 1974; Kelsay 1985) have been found to form insoluble complexes with calcium resulting in less calcium available for absorption. On the other hand, a high protein diet and a high sodium intake have been demonstrated to increase urinary calcium loss in adults (Worthington-Roberts 1981 b).

2.5.1 Calcium Nutritional Status

The habitual calcium intake of an individual has a profound impact on calcium absorption and retention (Hegsted, Moscoso & Collazos 1952; Malm 1958; Council on foods and Nutrition 1963; Beaton & Patwardhan 1976). Hegsted et al (1952) conducted a study on the minimum calcium requirements of adult prisoners. The urinary calcium excretion was found to correlate linearly with calcium intakes. It was concluded that a high calcium intake in the immediately past leads to a higher urinary calcium excretion and vice versa.

In fact, individuals with high plane of calcium nutrition subsist on high calcium diets, the absolute amount of calcium absorbed increases despite the fact that the rate of fractional calcium absorption reduces. However, a relatively large amount of unabsorbed calcium is excreted in the feces (Nordin & Marshall 1988). Heaney, Saville & Recker (1975) used radioactive technique to determine calcium absorption in adults and indicated that calcium absorption appeared to be 53% at an intake of 190 mg and dropped to 17% at an intake of 3,000 mg.

2.5.2 Vitamin D Nutritional Status

Vitamin D in natural food sources is mainly derived from animal food, for example, livers and fatty fish are rich sources of vitamin D. Vitamin D can also be synthesized under the skin when exposed to ultraviolet light under the sun (Worthington-Roberts 1981a).

In most climatic conditions, normal adults obtain adequate amount of vitamin D by exposure to sunlight. The amount of vitamin D formed is well correlated with the extent of exposure to ultraviolet light (Devgun, Johnson, & Paterson 1983). It was demonstrated that a few hours of exposure under the sun in summer was able to produce sufficient vitamin D to avoid deficiency for several months (Poskitt, Cole & Lawson 1979). Concern about dietary provision is unwarranted except under conditions of limited exposure to the sun (Davie & Lawson 1980).

Since vitamin D is required for intestinal calcium absorption, an adequate supply of vitamin D either from oral route or from sunlight exposure is important in infants and children. A recent study (Leung et al. 1989) on the vitamin D status of Hong Kong Chinese infants at 18 months indicated that the serum level of 25-hydroxycholecalciferol was normal and which was highly correlated with the global solar radiation, a higher serum value of vitamin D was found in summer months during which sunshine is abundant.

It is speculated that the body is capable of adapting to a habitual low level of calcium intake by increasing dietary calcium absorption and this adaptive mechanism required an adequate supply of vitamin D (Fraser 1988b).

2.5.3 Protein

Studies of Johnson, Alcantara and Linkswiler (1970); Walker and Linkswiler (1972); Anand and Linkswiler (1974) and Chu, Margen and Costa (1975) consistently demonstrated a remarkable relationship between urinary calcium output and a wide range of dietary protein intakes in young adult men. In these studies, subjects were given with calcium either at 500 or 800 mg/day, It led to nearly double the loss of urinary calcium when dietary protein was increased from 47 g to 142 g.

Furthermore, even calcium was given as low as 100 mg/day, the subjects on a high protein diet were all losing calcium in urine. However, enhanced intestinal calcium absorption was not observed in compensation for the significant rise in urinary calcium. It becomes obvious that high protein intakes adversely affect calcium nutrition status.

2.5.4 Phosphorous and Calcium to Phosphorus Ratio

The effects of phosphorus on the absorption and utilization of calcium have been a topic of continuing controversy. Phosphorus in the form of phosphates have been widely believed to reduce calcium absorption due to the the formation of insoluble calcium phosphate salts in the intestine. When rats were fed with diets high in phosphorus or diets low in calcium to phosphorus ratio (< 2:1), it led to bone resorption secondary to hyperparathyroidism (McBean & Speckmann 1974). The results from animal studies were unduly extrapolated into human situation. As a result, it is commonly believed that a low calcium to phosphorus ratio may have deleterious effects on calcium absorption and bone integrity. However, based on published data from human studies, Zemel (1985) and Allen (1982) do not support this hypothesis. In an often cited human study, Spencer, Kramer & Norris (1975) have shown that by varying phosphorus intake from 800 to 2,000 mg/day and the levels of calcium intakes maintained at 200, 800 or 2,000 mg/day in adult men, this did not result in any significant effect on calcium balance. In fact, the range of the calcium to phosphorus ratio in human diets may satisfactorily fall within this range of intakes (Worthington-Roberts 1981b).

2.5.5 Sodium

Recent studies have shown that increased sodium intake is linked with a rise in urinary calcium excretion in human (Sabto, Powell, Breidahl and Gurr 1984; Shortt, Madden, Flynn and Morrissey 1988) and in animals (Goulding 1980; Goulding and Campbell 1984). Data from Sabto et al. (1984) have shown that in adults an increase in sodium excretion of 1 g/day (2.54 grams salt) was associated with a rise in urinary calcium of about 26 mg/day. Shortt et al. (1988) reported that 24-hour urinary calcium excretion increased on average by 38.9 mg and 26.3 mg for one gram increment of sodium in men and women respectively.

Therefore, consuming a high salt diet over a long period of time may lead to negative calcium balance secondary to increased urinary loss of calcium.

2.5.6 Lactose

Lactose has been known for stimulating calcium absorption in animals (Evans & Ali 1967) and in human (Allen 1982; Cochet, Jung, Griessen, Bartholoÿdi Schaller, Donath 1983). Cochet et al. (1983) speculated that the increased calcium absorption in the presence of lactose of the lactase-normal group was due to the prolonged calcium transit time along with the sustained maximum rate of calcium absorption in the gut. Furthermore, it was noted that in both lactase-normal and lactase-deficient subjects, there was no significant difference in the mean rate of calcium absorption nor the intestinal calcium transit time when calcium was ingested without the concomitant administration of lactose. This suggests that the effect of lactose on calcium absorption is dependent on the existence of normal lactase activity. Therefore, lactose intolerance has a negligible effect on calcium absorption.

2.5.7 Glucose and Glucose Polymers

Studies over the last 15 years have indicated the effect of glucose enhanced calcium absorption in animals and in humans (Allen 1982; Zheng, Wood & Rosenberg 1989).

An administration of glucose and glucose polymers in vivo enhanced the fractional calcium absorption (Knowles, Wood, Rosenberg 1988; Zheng et al. 1989). A coadministration of 40 g glucose observed a 49% rise in the rate of fractional calcium absorption in the studied group than in the controlled group without giving glucose with the calcium dose (Knowles et al. 1988). The enhancement effect of glucose polymer was independent in the subjects with marginal vitamin D status (Zheng et al. 1989), results from these studies showed that glucose or glucose polymer enhances intestinal calcium absorption in man. A potential positive effect of co-administration of glucose in enhancing calcium bioavailability deserves further study.

2.5.8 Phytate

Phytate is a phosphorus storage compound naturally present in cereals, legumes and nuts.

Phytate has been identified as a potent calcium binding anion that will complex calcium in the small intestine, thereby reducing the availability of calcium for absorption and increasing fecal loss of calcium

(Reinhold 1976). However, phytate can be hydrolysed in the intestine by the action of phytase which is present in the phytate containing food, being synthesized by the intestine and intestinal microflora (Williams & Taytor 1985). Some cereals such as wheat and rye contain their own phytase. It has been estimated that 50% of ingested phytate phosphorous is absorbed in the intestine (Henry & Kon 1945).

The effects of phytate on calcium absorption has been identified in human in whom the main dietary source was bread and wholemeal flour. When the subjects were fed with a diet containing 92% extraction rate flour, calcium absorption was less efficient than when 69% extraction rate flour was used. The addition of phytate to the 69% extraction rate flour depressed calcium absorption further and this would be overcome by the addition of calcium (McCance & Widdowson, 1942).

Although phytate is known to complex with calcium, the view that it might lead to impaired calcium absorption has been challenged by studies which could not confirm such effect in man (Sandeberg, Hassalblad & Hassalblad 1982) or in animals (Graf & Eaton 1984).

2.5.9 Oxalate

Oxalate is found in spinach, rhubarb, beetroot, peanut, cocoa, chocolate and tea. However, little published information is available on the amount of oxalate in Chinese food.

It is well known that oxalate present in some plant food interferes with the bioavailability of calcium in animals and in humans (Mcbean & Speckmann 1974; Kelsay, 1985). Oxalic acid in diet may complex with calcium to form insoluble salts and reduce the availability of calcium for absorption. In human studies, there is no significant difference between a high oxalate or a low oxalate diet on calcium balance (Kelsay 1985). However, there is evidence that consuming oxalate along with high level of dietary fiber may adversely affect calcium balance in human (Kelsay 1985).

Chapter 3

Methods of Evaluating Calcium Nutritional Status and Bone Mineral Content

There are various possible methods for evaluating calcium nutrition status and bone mineral content. These methods will be discussed in the following sections.

3.1 Methods of Evaluating Calcium Nutritional Status

3.1.1 Blood Biochemistry

Plasma calcium concentration is narrowly maintained within the range of 2.25 - 2.6 mmol/l as it was mentioned in Chapter 2. A slight alternation of the plasma ionised calcium concentration will result in the release of PTH and vitamin D_3 to elicit a number of physiological responses in order to restore the plasma calcium concentration to normal. Therefore, the plasma calcium concentration is not a sensitive indicator for assessing calcium nutritional status in population study.

3.1.2 Total Body Neutron Activation

The technique of "Total Body Neutron Activation" is a sophisticated method to access body calcium content (Nelp, Palmer, Murano, Pailthorp, Hinn, Rich, Williams, Rudd and Denney 1970). The method involves irradiation on the whole body with known dose of neutrons, the isotope calcium-48 in the body will then be converted to calcium-

49 and there will be a release of gamma rays. The body is scanned in the whole body counter to estimate the body calcium content. Although the radiation to the body is low, the technique requires extremely elaborate equipment and only a few equipment in the world can perform this sort of measurement. Therefore, the technique is not yet widely available for population surveys.

3.1.3 Metabolic Balance Study

The approach of balance study is to determine the relation between dietary intake and output in urine, feces and skin. The lowest intake consistent with a positive calcium balance is interpreted as the requirement. However, most of these studies conducted in the past were frequently too short to allow complete adaptation to occur during the experimental period. The numbers of subjects were too small to be representative (Paterson 1979; Worthington-Roberts 1981b; Kanis & Passmore 1989).

Adaptation may take place in the body to maintain calcium balance on low calcium diet. This adaptation may take months to complete (Hegsted 1952; Malm 1958). Hence, the true requirement of calcium would better be determined in long term balance study. Calculations based on sudden decrease in calcium intake may escalate the actual requirement of calcium (Kanis & Passmore 1989).

In addition, to conduct balance study in free living individuals is a labour intensive, costly and tedious exercise. Errors incurred in collection of feces and urine would biase the results (American Academy of Pediatrics, 1978; McBean & Speckmann 1974). It seems to be difficult at this stage to admit normal young children to a metabolic ward over a prolonged period of time in order to carry out a well controlled and accurate balance study. Therefore, the use of balance study in determining calcium status in children was not considered in this study.

3.1.4 Dietary Assessment

The use of dietary assessment to determine food and nutrient intakes remains an important tool in evaluating nutritional status of population groups or individuals. The findings of dietary assessment are important as an aid in clinical assessment of patients, in studying the dietary habits of food consumption in individuals or groups in relation to physiological and biochemical variables, in epidemiologic study of diet in relation to health and disease, the formulation of recommended dietary allowances and national food and agricultural policies.

3.2 Methods of Evaluating Bone Mineral Content

3.2.1 In Vivo Measurement of Bone Mineral Content

Over 99% of total body calcium is found in the skeleton. Therefore, to develop methods in quantifying bone mineral content in vivo would provide a better tool in evaluation of body calcium status (Fraser 1988b;

Christiansen, Rodbrol & Jensen 1975).

The determination of bone mineral content by bone biopsy is impractical in routine clinical assessment and in population studies (Sorenson & Cameron 1967).

Visual examination of the image of bone in radiographic films was commonly used in the 1950's to determine bone mineral content. However, this technique does not provide quantitative measurement. The precision of measurement is poor due to quality variations in film exposure and development, and also due to inconsistent interpretations by different observers (Bland, Soule, Van Buskirk, Brown & Clayton 1969). Moreover, this technique is insensitive because it is unable to detect changes in bone mass before a 30% loss occurs (Lachmann 1955). Therefore, a more reliable and sensitive in vivo technique is required to detect small changes in bone mineralisation.

The developments of advanced techniques of radiogammetry single photon absorptiometry, dual energy x-ray absorptiometry and quantitative computed topography from 1960's to 1980's have provided an aid to determine bone mineral content and calcium content in the body by a non-invasive means. Apart from the evaluation of body calcium status, the measurement of bone mineral content has a variety of application, such as in setting up normal values of bone mineral content in populations (Mazess & Cameron 1974; Mazess & Mather 1974; Hsu, Chan & Leung 1987); an aid to confirm clinical diagnosis and to monitor the treatment of demineralisation diseases, for example, osteoporosis and rickets (Horsman, Gallacher, Simpson & Nordin 1977; Recker, Saville & Heaney 1977; Recker & Heaney 1977; Eyberg, Pettifor & Moodley 1986) and to observe the effects of certain drugs in bone metabolism, for example, steroids and growth hormone (Adinoff & Hollister 1983; Shore, Chesney, Mazess, Rose & Bargman 1980).

3.3 Review on Adopted Methods

In this study, the evaluation of calcium nutritional status and bone mineral content were determined by the techniques of dietary assessment and single photon absorptiometry. The details of the methods will be discussed in the following sections.

3.3.1 Single Photon Absorptiometry (SPA)

3.3.1.1 Introduction

In 1963, Cameron and Sorenson developed a technique with the emission of photons from a finely culminated monoenergetic Iodine 125 radionuclide source for the evaluation of bone mineral in peripheral cortical bone. This technique is termed Single Photon Absorptiometry (SPA). The technique of SPA was primarily developed to measure the mid-shaft of the radius where mainly comprises cortical bone. Also, there are no major problems of surrounding fat and soft tissue in this region during measurement (Sorenson & cameron 1967).

3.3.1.2 The Principle

The principle of SPA is based on the investigation that when a beam of well collimated photon passes beneath a piece of bone to be scanned. A collimated scintillation detector, which counts the attenuated photons, is placed over the bone (see figure 1). The scintillation detector and the Iodine-125 source are moving simultaneously at a constant speed across the bone, the amount of photon absorbed by the bone mineral is different from that absorbed by the surrounding soft tissue. The attenuated photon counts are plotted by the computer in the form of absorption curve. The computer integrated surface area of absorption curve is proportional to the amount of bone mineral content (BMC) in the section of bone being scanned (Cameron, Mazess & Sorenson 1968).

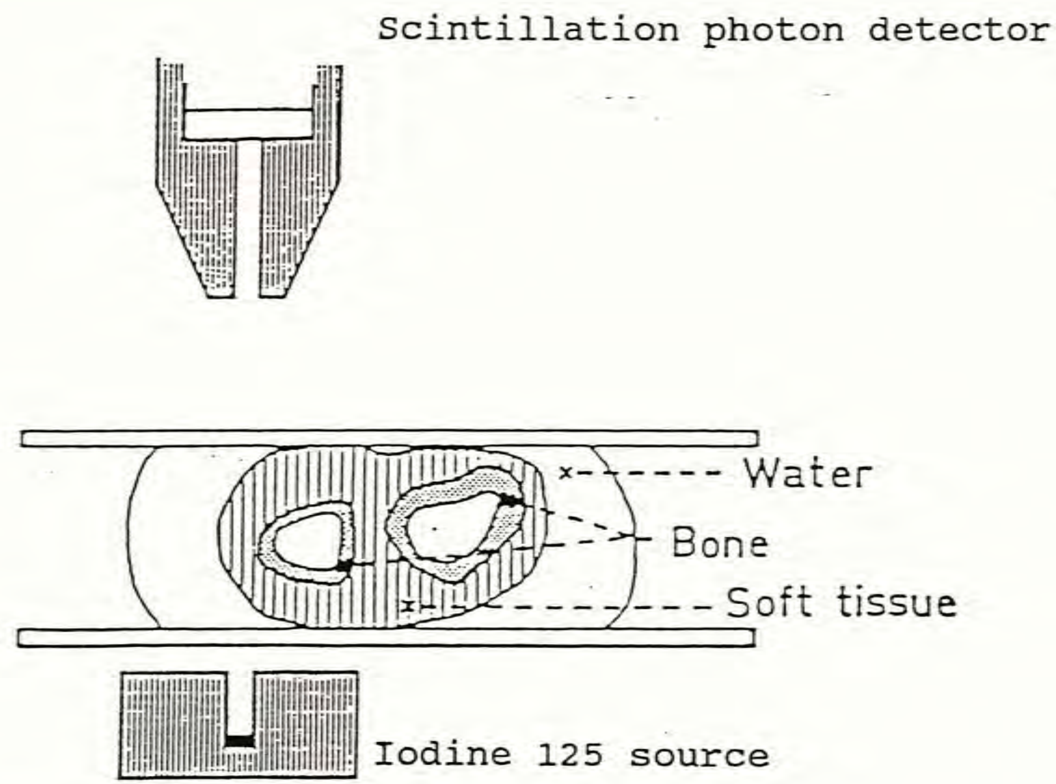

Figure 1 An illustration of bone mineral measurement by single photon absorptiometry

The unit of BMC is gram per centimeter, representing the mass of mineral in one centimeter of longitudinal section of bone. The length of the scan path, expressed in centimeter is proportional to the width of the bone. The BMC/BW or often referred as "bone mineral density (BMD)" is a ratio of BMC to BW, this value yields a measure of mineral mass per unit area. In many situations, the parameter of interest is bone density. Neither the BMC nor the BMD measures the actual bone density, because both the depth and the volume of bone cannot be determined by this technique. In addition, the area of the bone scanned includes the medullary cavity, the size of the medullary cavity may affect the measurement of bone mineral. Therefore, the technique of SPA does not provide an absolute quantity of bone mass. However, it provides some relative measures of bone mass for comparison purpose.

The parameter of BMD is less preferred than BMC to express the quantity of bone mineral, because the growth of cortical bone does not seem to be proportional to the growth of bone width. The medullary cavity in growing children is found to enlarge at a different rate from the growth of total compact bone thickness (Mazess & Cameron 1971). Ann Prentice and co-workers also observed that a linear relationship between the growth of BMC and BW in small children does not exist (Ann Prentice, personal communication). Therefore, BMD is not a good indicator of reflecting bone mineral mass due to non proportional increments in BMC and BW. Whereas the BMC of the distal forearm is a better estimate of total body calcium (Christiansen & Rodbro 1975).

3.3.1.3 Accuracy and Precision

The in vivo measurement of BMC by the technique of SPA has been reported to be highly correlated with bone mineral content of the excised bone being scanned. The coefficient of correlation varies between 0.96 to 0.99 (Sorensen & Cameron 1967; Christiansen Rodbro & Jensen 1975; Christiansen & Rodbro 1975; Mazess 1971). Christiansen et al (1975) showed that the calcium content in the excised bone has a high correlation with the measured BMC by the technique of SPA ($r = 0.991$). The precision in day to day variation was reported to be 2% in adults and 3 - 4% in children (Sorensen & Cameron 1967; Christiansen et al. 1975; Mazess 1971).

3.3.1.4 The Skeletal Site for Measurement with the Technique of SPA

The distal forearm particularly the radius, is frequently referred to be an appropriate site for BMC measurement with the technique of SPA. Measurement of the distal forearm may minimise the error incurred from various thickness of fat tissue in the body (Sorensen & Cameron 1967; Christiansen et al. 1975). Christiansen & Rodbro (1975) demonstrated that BMC in the distal forearm is well correlated with the total body calcium. Cohn, Ellis, Wallach, Zanzi, Atkins & Aloia (1974) compared the value of total body calcium measured by total body activation analysis and the value obtained from BMC measurement of the forearm, The correlation coefficient was as high as 0.97. Therefore, the BMC at the forearm is a reasonably accurate estimate of total body calcium. In addition, the forearm is a convenient site for measurement, the site is easy to relocate for repeated measurement. Thus, increases the reproducibility of measurement.

3.3.1.5 Radiation Exposure

The scan is localised at a thin cross-section of the radius and it will not expose the rest of the body to radiation because the photon beam is highly collimated to about 3.0 mm in width and the beam is almost completely absorbed by the detector.

3.3.2 Review on Methods in Dietary Assessment

3.3.2.1 Introduction

An accurate assessment of nutrient intake of free living individuals is a difficult and labour intensive exercise. There have been a number of different dietary assessment methods developed for use. The selection of a most appropriate method depends on the objectives of the survey, the accuracy required for the nutrient variables concerned and resources available. Different methods have their own advantages and inherent limitations. There will be a discussion on the choice of method in assessing dietary calcium intake in the present study.

3.3.2.2 Food Weighing Method

Widdowson (1936) was the first to use weighed food records to document food intakes of free living individuals. The principles of this method have been extensively reviewed by Marr (1971). During the study period which usually lasts for seven days (Widdowson, 1936), the subjects are asked to weigh and record any individual food items immediately before eating. Edible food left over is also weighed and recorded. Cooked food that is weighed immediately before eating is a typical feature of this method.

Nowadays, the subjects in this kind of survey are usually provided with a digital electronic scale with a tare button for food weighing. Individual food items are weighed before eating. With the tare button, the subject can read out directly the weight of an individual food while conducting additive weighing without any calculations (Marr 1961; Marr 1965). Thus, it avoids additions and subtractions. As a result of using digital electronic scales in weighed intake survey, the accuracy of weighing records has been improved (Bingham 1987).

Since the subjects in this kind of dietary survey need to self-administer the entire procedures of food weighing and recording, a clear understanding of the method prior to the study is important. Therefore, instructions and practices of weighing and recording are usually arranged for the subjects prior to the study. Furthermore, sufficient supervision during the study is a prerequisite for obtaining accurate and reliable data. Hence, home visits are normally required during the study period to ascertain that the procedures are conducted properly (Bingham, McNeil & Cummings, 1981). In addition, maintaining usual dietary habits throughout the study is emphasised to the subjects (Marr 1971).

In weighed intake survey, in order not to interfere with normal dietary habits of the subjects, some of the records given with only full descriptions of the amounts of food eaten are usually accepted for occasional meals eaten away from home (Bingham, Cummings & McNeil, 1979). Whenever possible, the weight of food will be estimated by a nutritionist at a later stage by purchasing and weighing a similar food portion from the restaurant or shop where the subject obtained the food.

In addition, composite dishes with mixed-up ingredients are difficult to weigh out separately. For example, with a piece of home-made apple pie, the subject will be instructed to weigh out the raw ingredients, namely, flour, margarine, sugar, apple and water used in making the pie. A precise portion or the average portion of the cooked pie taken by the subject is weighed before eating. The nutritionist will then be able to evaluate the weights of food ingredients eaten by considering the total number of portions and the weights of raw ingredients used in preparing the pie.

Marr (1971) stated that a 7-day period would appear to be adequate to carry out weighed intake survey. A period less than 7 days might underestimate the daily variations. And if the study period is more than 7 days, there might be a lack of cooperation and motivation from the subjects. McCance, Widdowson & Verdon-Roe (1938) commented that statistically speaking, a week is quite sufficient to distinguish a big eater from a small eater.

There are limitations in weighed intake method. As a subject is asked to weigh and record what he eats, there is always a risk that the subject may alter the eating habits, either subconsciously or to simplify the task or because he is made aware of the quantity and quality of what he eats or he tries to impress the researcher (Bingham 1987; Morgan, Jain, miller, Choi, Matthews, Munan, Burch, Feather, Howe & Kelly 1978). Trulson & McCann (1959) strongly stated that the weighing of food would alter intake. Huenemann & Tunner (1942), and Yudkin (1951) concluded that a single weighed intake survey cannot be considered "typical" of a subject's food intake over a long period of time. Ohlson, Jackson, Boek, Cederquist, Brewer, & Brown (1950) reported that food intake was higher when measured by three 24-hour recalls as compared to 10 days weighed intake. The authors attributed the low intake to the reduced dietary intake from the subjects in order to avoid such time consuming procedure. Van Staveren & Burema (1989) also commented that the use of weighed intake method for observational studies in large population is too cumbersome and time consuming.

3.3.2.3 Food Recording Method

Youmans, Patton & Kern (1942) were the first to measure individual food habits in a group by using standard cups, teaspoons or tablespoons, etc. The volume of these measures were standardised with a reference table. The amount of food such as bread, cake, meat, and biscuits, etc., were determined by measuring their dimensions. Fruits and vegetables were taken down either raw or cooked. Whereas the sizes of apples, oranges, potatoes and tomatoes, etc., were designated as large, medium, or small by referring to the standard portion size described in food composition tables.

Nowadays, an introduction to the method in recording intake will be given prior to the study. The subjects will be given with standard record forms for food recording at home. Home-visits are arranged during the study period to ensure that the recording procedures are carried out properly (Marr, 1971).

McHenry, Ferguson & Gurland (1945) assessed the reliability of the 7-day record for every first week over a 12 months period. The authors indicated that there were substantial variations in nutrient intakes which was attributable to personal and seasonal factors. And suggested that a 7-day record was less reliable in populations with free choice of food. The subjects participate in this sort of dietary survey, like those involved in the weighed intake method, their eating habits might alter because of being aware of their food habits (Marr 1971). The errors incurred in describing the size of food and recording mistakes are always implicated in this sort of dietary survey (Bingham 1987).

3.3.2.4 24-hour Recall Method

This dietary assessment is carried out during an interview with the subject. The subject will be asked to give detailed descriptions of food and drinks that he took in the previous 24 hours (Marr,1971). In assisting the estimations of food consumed, food models and general household measures such as various sizes of cups, glasses, bowels and spoons etc., will be on display during the interview. The size or portion of any food item is reported in general household measure or by referring to the size of a food model which is closely resembling the food.

Bingham (1987) compared the magnitudes of variations in mean energy intake as measured by 24-hour recalls and by other reference methods, for example, a 7-day food recording method. The coefficients of variation varied from seven to 40% as quoted in the literature. It was concluded that the 24-hour recall method gives unacceptable large errors to either food or nutrient intake.

The method of 24-hour recall has been consistently found to under estimate food intakes (Trulson 1954; Acheson, Campbell, Edholm, Miller & Stock 1980; Bull & Wheeler 1986).

Bingham (1987) reviewed the day to day variation of calcium intake in individuals, the coefficient of variation was about 35% over a seven-day period. It might require three to 15 replicate observations to estimate usual intake with statistical confidence in 24-hour recall.

Linusson, Sanjur & Erickson (1974) validated the 24-hour recall method with food weighing technique. They concluded that the 24-hour recall method is fairly accurate for qualitative estimation of mean intake of a group. And the method was not accurate for estimating food in quantity.

Despite the fact that the 24-hour recall is of limited value in characterising individual dietary habits and also it is not an accurate estimate of usual intake. The single 24-hour recall can be used if the sample size is relatively large.

In population based observational study, the effects of random errors incurred in the 24-hour recall can be reduced by increasing the numbers of observations in the study. (Van Staveren & Burema 1989). The 24-hour recall method has been used extensively in large population based studies due to its simplicity and shorter time for recording (Van Staveren & Burema 1989).

3.3.2.5 Food Frequency Method

Wiehl & Reed (1960) were the first to suggest the use of questionnaire in epidemiological studies of cardiovascular disease. This questionnaire consisted of a short list of selected food items. The record was only an qualitative estimate of variation in eating habits. The results of food intakes were rated into four to five categories to identify the big and small eaters. No attempt was made to quantitate food intake. The authors believed that if a group of individuals could be differentiated by "use" or "non-use", "frequent" or "infrequent" use of selected food on the list, then such characteristics could be tested for disease association.

The two remarkable features of food frequency questionnaire are its simplicity and requiring shorter period of time for obtaining dietary information. Stefanik & Trulson (1962) described a questionnaire lasting 20-35 minutes in which nutritionists asked subjects how often they ate 41 items of food. Abramson, Slome & Kosovsky (1963) included questions on both frequency of consumption and amounts of food with an aid of household measures and food samples in a 30-minute interview.

A number of questionnaires have been subsequently developed either self-administered by the subjects or completed by interviewers at a short interview (Bingham,1987). Nowadays, a lot of questionnaires have been devised to estimate intakes quantitatively, using food models (Epstein, Reshef, Abrahamson & Bialik 1970) or photographs (Hankin Rhoads & Glober 1975; Jain, Harrison, Howe & Miller 1982) to assess portion size.

Cummings, Block & McHenry (1987) compared calcium intake obtained from seven-day records to those obtained from a frequency questionnaire using five, ten or 15 food items high in calcium content. Correlation coefficients were 0.76, 0.75 and 0.67 respectively. However, this study was conducted in an American population whose calcium intakes are largely contributed from the use of milk, the inclusion of multiple questions on milk would represent a large percentage of calcium intake. Johnson (1989) needed to include a list of 18 items of food to estimate calcium intake in Asian-American and she concluded that the Asians rarely consume as much dairy products as the Caucasians and they obtain calcium from non-dairy food. Therefore, an indiscriminate use of food frequency questionnaires from a population whose dietary practice is different from the studied group would certainly give erroneous results.

Food frequency questionnaires have been the most frequently used dietary assessment method in large scale epidemiological surveys. Quantitative estimation of their accuracy have not been successful because food frequency methods are primarily designed to assess the intake of a specific nutrient or food, other than the intake of all nutrients (Bingham 1987). Attempts to assess individual intakes quantitatively by questionnaire have been unsuccessful as well. Epstein et al. (1970) reported that a correlation of 0.57 or less between a questionnaire and a standard dietary history for individuals with varied diets, and overall the questionnaire underestimated energy intake by 27%. The workers commented that their questionnaire was not suitable for evaluation of quantitative dietary intakes.

Abramson et al. (1963) reported correlation coefficients of 0.41 and 0.61 for two food items when comparing the results obtained from food frequency method and food recording method. The food frequency questionnaire of Browe, Gofstein, Morlley & McCarthy (1966) gave consistently lower average intakes when the results were compared with a standard diet history administered by a nutritionist. Stuff, Gorza, Smith, Nichols & Montandon (1983) commented that there was a poor agreement between results of calcium intake obtained from food frequency questionnaire and those from a seven-day food record because correlation coefficients for dietary intakes ranged from 0 to 0.24. Yarnell, Fehilly, Milbank, Sweetnam & Walker (1983) stated that food frequency questionnaire would not completely replace the need for detailed studies of individuals in epidemiology. Correlation coefficient between their questionnaire results and those from a Seven-day weighed record for the nutrients assessed ranged from 0.27 to 0.41.

In conclusion, a lack of agreement between measured dietary intake and that estimated from questionnaire has been a consistent finding in using food frequency method (Bingham 1987). And there is no short cut method of dietary assessment of dietary intakes of individuals (Bingham 1987).

3.3.2.6 Dietary History Method

Burke (1947) was considered the first to use dietary history method as a tool in the assessment of dietary intakes. During an interview, the respondent was asked about the usual eating patterns, both at meal times and in between meals, and the food consumed was recorded in common household measures. Questions like "What do you usually eat for breakfast?" And followed by further questions to include daily variations until a complete picture of usual food taken at breakfast was obtained. The portion sizes of food were all recorded in household measures. The interview continued in this way until a comprehensive record of usual daily intake with its variations were carefully recorded.

After the dietary history record has been completed, the record was cross checked with a list of various food groups. The subject was asked about the frequency and the amount of a particular food taken over a specified period of time. Burke (1947) commented that the accuracy of the method is greatly improved with a final cross check.

Reed & Burke (1954) measured the reliability and validity of a dietary history taken on 103 children under 6 years of age. They concluded that dietary history technique is useful for determining average levels of nutrient intakes.

Bingham (1987) commented that the dietary history offers a major advantage over recording or recalls covering a long period of time, if an estimate of usual diet can be obtained at one setting, such that the errors incurred with time variation (i.e. daily, weekly, or seasonal variation) will be eliminated.

Huenemann & Turner (1942) undertook a dietary survey with 21 adolescents and children, these subjects kept weighed intake records for six weeks and the nutrient intakes obtained from the average of the weighed intake records were compared with the results from a dietary history. The authors commented that a six week period with the weighed intake method was sufficient to validate the dietary history method that is used to establish habitual intakes of individuals. The coefficients of variations between the two methods were 11%, 16%, and 19% for energy, protein and calcium respectively. The regression coefficients of energy, protein and calcium were 0.50, 0.7 and 0.97 respectively. Therefore, the dietary history method is reasonably accurate in assessing the habitual intake of an individual.

There have been few comparative studies between the method of dietary history and weighed intake or food recording method. And very few studies were carried out long enough to determine the accuracy of the method of dietary history which was primarily designed to determine the habitual intakes of individuals. Moreover, the alternation of dietary habits during the period of food weighing or recording needs to be identified, probably by using biological markers to correct the difference, before any meaningful validity of the dietary history method can be made (Bingham 1987).

3.3.2.7 Chemical Analysis of Duplicate Diet

The chemical analysis of duplicate portions of food consumed by a subject can give the best measure of dietary intake. The subjects are provided with a number of containers and asked to keep an exact, weighed duplicate of every thing consumed for a specified period of time, A record of the weight of every food item was kept by the subject. During the study, home visits are necessary to resolve any problems that might arise. However, results from the analysis of duplicate diet consistently found to be lower than those of daily recall, weighed intake and food frequency interview (Bull & Wheeler 1986).

Duplicate collection of food has been criticised as more burdensome than just weighing food, and this method is suitable for controlled metabolic studies which examine the effect of nutrient intake on physiological outcome over a period of time. Moreover it does not reflect the usual eating habits of an individual (Bingham 1987, Akesson, Johansson, Svensson, Ockerman 1981).

3.3.2.8 Dietary Assessment by Photographic Method

The assessment of food intake by taking photographs at meal times was suggested by Elwood & Bird (1983). A camera is set up at the subject's home, photographs were taken in front of the subject who is ready to eat.

The authors commented that it is a simple method for the studied subject. A set of reference pictures with various sizes of food portions corresponding to the image of the food in the photograph was used to estimate the weight of food consumed.

However, the authors also noted that using this method in isolation cannot tell the cooking method, the use of sugar in tea, and reconstitution of beverages, etc. Besides, it cannot assess food intake when the subject is eating away from home. Therefore, it is not suitable for use in free living individuals.

In addition, it is expensive to use in any large observational surveys, labour intensive and time consuming in moving and setting up the equipment at the subject's home. Furthermore, training field workers and the preparation of reference pictures for food portion evaluation is tedious. Hence, this method has not been extensively used in large scale surveys.

3.3.3 Dietary Assessment Methods Adopted in the Present Study

The purpose of dietary assessment in the present study was to estimate quantitatively the habitual dietary intake of calcium in a group of pre-school children. Vitamin D status may have profound effect on bone health in children. However, vitamin D status has been found normal in Hong Kong young children because most of the children obtain adequate vitamin D from exposure to the sun (Leung et al. 1989). Thus, vitamin D was not assessed in the present study.

Beaton (1989) in his recent article concluded that "There is no perfect dietary methodology. However, there are preferred methods for defined purposes. The task is to match method and purpose." From the limited information reviewed above, one may conclude that any single method has its own benefits and limitations. Unbiased estimation of dietary intakes seem to be unobtainable (Bingham 1987). In order to select the most appropriate method for use, one has to consider the objectives of the study, the accuracy of nutrient estimation, and the human and financial resources available.

An excellent comparative study on dietary survey methodology (Bull & Wheeler 1986) suggested that any single method used in isolation to assess food consumption or nutrient intake of a group of individuals, there is always a probability of over estimating or under estimating intakes. Therefore, a combination of different methods in a dietary survey may provide a more valid and balanced estimate of habitual intake in individuals. Hence, it was decided to use a combination of three different methods to accurately assess the dietary intakes of calcium, energy and protein in the pre-school children.

In order to quantify nutrient intake established persistently over a longer period of time, the method of "dietary history" would be a better choice among other methods. The results obtained from the method of dietary history in comparison with 24-hour recall or food frequency method have been found to correlate better with those obtained from standard weighing or recording methods. Dietary history allows day to day variation and seasonal variation in food intake. The speculated criticism that the results would be bias because respondents are easy to report incorrect information during interview. In fact information can be cross checked with food frequency check list and/or 24-hour recall.

Although the methods of weighing or recording food are actually measuring what the subject eats during the study, the method of dietary history would be superior to food weighing or recording methods. Dietary history does not require cumbersome weighing and recording procedures. Thus, it reduces the possibility of alternation in food habits or under-report of food intake by the subjects in order to simplify the task.

Furthermore, to conduct dietary survey with the technique of weighing or recording in a broad spectrum of socio-economic classes might encounter practical difficulties. It is better to carry out in subjects with literacy to avoid unreliable records. The motivation and cooperation from the subjects to carry out a complicated and tedious dietary assessment might be lowered, particularly if some mothers have to look after more than one young child in the family or those who have a full-time job. It is unlikely that they will be willing to perform such a time consuming procedure in a period not less than seven days.

An additional advantage of dietary history is that it requires little prior training and home visits, it reduces tremendous constraint on labour resources in any single handed project.

Moreover, in order to obtain an accurate estimate of calcium intake, the use of a quantitative food frequency method as a supplementary method might give a more valid estimate of calcium intake. Finally, a cross check with 24-hour recall would increase the accuracy of the information obtained (Burke 1947).

3.3.4 Food Composition Tables

Nutrient analysis from most dietary surveys is dependent on food composition tables, unless food taken is analysed chemically. The quality of food composition tables affects the accuracy of nutrient estimation. Ideally, each country or region should have its own food composition tables comprising most of the food available in the region, either locally produced or imported food. Nutrient values from food tables that have been published for use in different countries are inconsistent among themselves (Bingham 1987). It is due to geographic variation in soil mineral contents, difference in species of plants and animals, variation in breeding and feeding live stock, different means of sampling procedures and chemical analysis, etc. An indiscriminate use of foreign food tables to analyze nutrient contents in local food might incur errors in result. For example, the flour used in the United Kingdom is fortified with calcium (Paul & Southgate 1978), the use of a British food composition table to calculate calcium content of bread baked in Hong Kong would over estimate its calcium content.

In Hong Kong, published local food composition table is not yet available. As most of the food in Hong Kong are imported from abroad, mainly from China, to compile a food table with nutrient data derived from other well established food tables for use in Hong Kong is necessary at this stage until a local food table is available.

The selection of appropriate food items for the compiled food table requires careful consideration. One should be aware of the places of origins of the imported food, the characteristics of agricultural practices in those countries, the sampling procedures and the methods of chemical analysis applied in the food tables.

In addition, most Chinese food or dishes may not be available from foreign food tables, e.g., dim sum, barbecued pork, shark fin soup and rice congee, etc. Therefore, it is necessary to include the nutrient values of these typical Chinese food commonly used in Hong Kong.

The Growth and Nutrition Research Team at the Department of Paediatrics, The Chinese University of Hong Kong has complied a food table for analysing dietary data. The table is based on food tables from Britain (Paul & Southgate 1978), Canada (Bowes & Church 1970), China (Institute of Health, Chinese Academy of Medical Sciences, 1980), Taiwan (Tung, Huang & Li, 1961), and the U.S.A. (Watt & Merrill, 1983; U.S. Dept. of Health, Education and Welfare 1972). In addition, chemical analysis of some common Chinese food by the government chemist, and product information from food manufacturers are also included in the table.

Chapter 4

Subjects, Materials and Methods

4.1 Subjects

In 1984, the Department of Paediatrics of the Chinese University of Hong Kong commenced a longitudinal study in Shatin which is a new town in the New Territories of Hong Kong. This study aims to investigate the state of health, patterns of growth and nutritional status of normal Chinese infants. The subjects of the present study are the cohort children of the aforementioned longitudinal study.

In 1984, 174 normal Chinese infants (94 boys and 80 girls) born at term to healthy mothers were randomly selected as the mothers brought the new born infants to a Maternal and Child Health Clinic in Shatin for the first routine check up after delivery. In order to select a sample representing different socio-economic groups and to avoid over representation of infants coming from the lower socio-economic classes, subject selection was by means of stratified sampling according to the types of housing (Goldstein 1986). In Hong Kong, about 50% (2.8 million) of people are living in government subsidised public housing which accommodates for lower income families (Hong Kong Government Information Service, 1990). For those who lived in public housing, one in three was selected, if they came from private housing, one in two was selected (S.S.F. Leung, Personal communication). Table 1 shows the types of accommodation of the study infants' at the time of recruitment in 1984.

In Hong Kong, a majority of mothers are aware of the public health care service provided by the Maternal and Child Health Clinics for their new born babies and themselves after delivery. These clinics are distributed all over the territory. Over 92% of the new born babies are routinely followed up at one of these clinics (Hong Kong Government Information Services, 1986). Therefore, these clinics are ideal centers for subject sampling in observational surveys.

The average age of the infants at recruitment were seven days. In the subsequent years the infants have been seen on regular basis. At each follow-up, a detailed dietary assessment, anthropometric measurements, selected biochemical and clinical investigations were carried out.

Until 1988, there were 141 cohort children at the age of four remained in the longitudinal study. The cooperation rate (81%) has been excellent. The parents of the studied babies have been very cooperative, enthusiastic in reporting dietary information of their babies. The parents were well trained in reporting dietary information by the research dietitian in the cohort study. The research dietitian has never tried to influence the eating habits of the studied babies (personal communication with the Growth and Nutrition Research Team, Department of Paediatrics, The Chinese University of Hong Kong).

Table 1

Types of Accomodation of study families

Type	n	%
Low cost housing	71	40.7
Home ownership housing	25	14.3
Private housing	40	23.0
Village	23	13.2
Quarters (Provided by employers)	10	5.7
Temporary housing	5	2.9
	174	100.00

Furthermore, the accumulative dietary records of the studied children since birth have already been collected. This information would be useful in studying the long term effects of dietary intakes on bone mineralisation in children.

During their latest follow up in 1988, over 95% of the parents expressed their willingness to return again in 1989 when the children became five years of age. Having taken into consideration the characteristics of this group of cohort children, it was decided that this cohort would be suitable subjects for the proposed project in studying calcium nutritional status in relation to bone mineral content of local pre-school Chinese children.

In order to obtain the current socioeconomic background of the study families, the occupations of the fathers were classified in accordance with "The classification of employed persons by occupation" in the quarterly report of general household survey (Hong Kong Government Census and Statistics Department 1990).

4.2 Weight and Height Measurements

Nude weight was measured on an electronic body weight scale (Seca delta model: 707) accurate to the first decimal place. Body height was measured without shoes on by a Harpenden stadiometer.

4.3 Dietary Assessment

The author in the present study was solely responsible for conducting dietary interviews with the parents. The respondent to the interviewer would be ideally the mother who in general should know well the child's food habits. If the mother could not come with the child, the father or the grand parent (living with grand parents is still common in some Chinese families with the traditional concept of extended family) who is next to the mother in the family knowing the feeding habits of the child would be invited to the interview. Any uncertain or unclear answers during the interview would be clarified by contacting with the mother at a later stage.

As discussed earlier, any single method of dietary assessment has its inherent limitations (Bingham 1987; Beaton 1989). It has been suggested that a combination of methods would provide a more valid estimate of the habitual intakes in a group of individuals than would any single method (Bull & Wheeler,1986). Therefore, in the present study, a combination of three different dietary assessment methods were employed. These methods were: (a) dietary history record, (b) food frequency record and (c) 24-hour recall.

During each interview, a standard record form was used for keeping the dietary record of each child using the three methods. A copy of such a record form is shown in Appendix I.

It was difficult for the parent to recall detailed food intake of the child in the whole year. Thus, the parent was asked to give the frequency and amount of food usually eaten within the two months before the visit.

4.3.1 Dietary History Record

The dietary history record form (In section I of Appendix I) has been arranged in the order of breakfast, lunch and dinner. This arrangement would allow the parent to focus on reporting food intake at each of the three main meals of the day that supply most of the essential nutrients. And then followed by reporting intakes of fruit and fruit juice, snack food and occasional fast food which might be taken in between meals.

Another feature to note in the dietary history record form is that it does not provide a list of food items that cover, if possible, an entire range of food that are commonly available in Hong Kong. The interviewer has got a good knowledge of the dietary food habits of Hong Kong Chinese. In fact, his questions on food consumption were based on the classification of different food into five categories, namely, (i) bread and cereals group, (ii) meat, poultry, fish and egg group, (iii) milk and milk products group, (iv) vegetable and fruit group, and (v) fat and oil group. The parent was guided through the five food groups, and reported the food eaten by the child as represented from each of the food groups. This facilitated an systematic report of food intake and might avoid under or over-reporting due to confusion.

The dietary history interview started with a typical question like "How often does the child take breakfast?" This question required the parent to indicate the frequency of breakfast taken. Probes would be asked if the parent gave vague answer such as "sometimes", "quite a lot" or "rarely", etc. The next question would be "What does the child usually eat for breakfast?" The parent was expected to give a list of food items usually taken by the child at breakfast. Then the reply might be, say, "He has bread, noodle and congee ..." It was necessary to clarify what kinds of bread, noodle, and congee as there is a wide variation for each food. The parent might reply and indicate that the child had sliced white bread, sandwich, pineapple bun, instant noodle or rice noodle in soup for breakfast on the days he had at school. And he would prefer beef congee and fried noodle during week ends. The interviewer needed to explore further the kinds of spreads or stuffings in bread or in sandwich. And the kinds of meat, fish, egg and/or vegetables, etc., ingredients that might be cooked with the noodle or congee. The cooking methods for preparing these food were also requested.

Furthermore, the kind of beverages that the child might drink at breakfast was asked. If a child usually had a glass of hot milk with Horlick for breakfast, the type of milk (either fresh milk, evaporated milk, sweetened condensed milk, or milk powder, etc.) and whether sugar was added were asked. If the answer was a glass of orange juice, then it needed to clarify whether it was pure orange juice or orange drink. If it was orange juice, then whether the juice was squeezed from fresh oranges or a commercial product, whether sweetened or unsweetened, required reconstitution or not.

After obtaining a list of food items at one setting, the parent continued on reporting the amount of food usually consumed by the child at the meal. An array of household measures, including different sizes of spoons, cups, glasses and bowls were on display to facilitate the description of the amount of food which was usually eaten by the child. The parent would be asked, "How much does the child usually have that food?" or "What would the child's usual portion be ?" The amount of a particular food was expressed in terms of teaspoonful, tablespoonful and cupful. Alternatively, some food might be conveniently described by its size or dimensions, for example, sausages, sliced bread, fruits or dim sum, etc. The number of helpings of the food taken at each meal was also recorded.

For any composite dishes, the recipe, the amount of raw ingredients used, the cooking method, the total number of portions derived from the dish and the description of the portion eaten by the child would be obtained. This enabled the author to evaluate the amount of individual ingredients eaten from the dish. For food eaten away from home, the descriptions of food portion and the cooking method were asked. If the interviewer felt that the information given by the parent was not sufficient, he might request the name of the food outlet or restaurant in order to buy a similar food portion for weighing or descriptions at later stage.

It would be meaningful to record usual food intake if a particular food item is taken at least once a month (Jain 1989). Therefore, if food was taken less than a month it would not be considered as important. The parent was asked "How often does the child have that?" Any answers with statements like "occasionally" or "several times a week" that did not quantify the idea of frequency would require further answers until the parent determined the frequency in terms of numerical answers. The interviewer never assumed or suggested frequency of a particular food eaten. The parents have never been rushed to give an estimate of frequency, they were given sufficient time to think carefully the frequency of food intake.

There would be some children who might consume a wide variety of food from time to time. It would be difficult to establish a usual eating pattern in these children. Hence, the interviewer would need to probe with question like: "On the basis of last month, how often would you think the child eat that...?"

The frequency of a particular food item taken at each setting was recorded as the number of times consumed per week, per fortnight or per month (30 days).

4.3.2 Food Frequency

The selected food groups listed in the food frequency record form (Appendix I Section II,) are the major sources of calcium in diet. The food frequency record aims at estimating calcium quantitatively from calcium rich food. Furthermore, the categorization of different sources of calcium into selected food groups facilitated systematic reporting and recording by the parent and the interviewer respectively. Moreover, reporting food items eaten in respect to these selected food groups would minimise the risk of missing out any food items which might constitute a substantial source of dietary calcium for some children. A record for the use of calcium supplement is also given in this section.

The time frame for recalling food in the frequency record was also two months immediately before the interview.

Milk contains a rich source of calcium. Therefore, milk brand, strength of reconstitution, volume, and frequency of drinking were recorded for detailed calculation of calcium intake. For milk powder reconstituted differently from the manufacturer's specifications, the parent was asked to give the number of scoops or tablespoons of powder used in reconstitution.

The descriptions of portion size and the frequency of consuming a particular food on a daily, weekly or monthly basis were reported in a similar approach as described in the section of dietary history. For example, when a parent was asked about the usual intake of dairy products. The first question would be, "How often will the child take dairy products, namely, cheese, ice-cream, yoghurt, etc." It would request the parent to think about the relevant food items in each food group.

At the same time in order to stimulate the parent to recall the relevant food items that the child usually consumed, the interviewer would list out different varieties of food in each selected food group.

4.3.3 24-hour Recall

Having completed the dietary history taking and food frequency record. A 24-hour recall of food intake on a typical day was reported by the parent to cross check the information given in the dietary history as well as the food frequency record. The parent was asked to recall any food eaten by the child in between the time he got up in the morning until he went to bed at night on a typical day in the last two months. The parent was also asked to recall the specific time and place that the child consumed the food. In this way the report of food intake was in chronological order which reminded the parent that at certain time of the day the child might consume certain food. Household measures continued on display to assist food description.

4.3.4 Estimation of the Amount of Food

The weights of some local food in various portion sizes commonly used by children were determined by weighing in the laboratory using a digital electronic scale (Mettler model PC 2200.), accurate to 0.1 gram. These served as average reference portion weights. The amount of food described by the parent was estimated by determining the size of the described food portion as how it differed from the reference average portion size. Alternatively, some of the food described by the parent might be estimated by referring to the standard portion size as described in the food tables employed.

4.3.5 Nutrient Analysis

Dietary information as collected by the methods of dietary history, food frequency and 24-hour recall were integrated for nutrient analysis. The dietary assessment obtained by the method of dietary history provided qualitative and quantitative information of food intake over a persistent period of time. Therefore, it was the main body for nutrient estimation. In addition, the method of food frequency was used to validate the quantity and frequency of food intake as reported in the dietary history. And the 24-hour recall was used to check if there was any major day to day fluctuation in food consumption. If there was discrepancy occurred among the dietary data obtained by these three methods, their mean value would be considered. In the present study, such discrepancy was rarely encountered. At most, the difference would be like : three bottles verses four bottles of fresh milk consumed in a week, or one tablespoon verses two tablespoons of green vegetable taken at a particular meal. Perhaps, the parents in the longitudinal study have been well trained to report accurate dietary information. Therefore, the estimation of dietary intake was basically obtained by the method of dietary history with occasional modifications from food frequency and 24-hour recall.

The food composition table compiled by the Growth and Nutrition Research Team as mentioned in Section 3.3.4. was used in analysing nutrient values of energy, protein, fat, carbohydrate and calcium in food. The accuracy and reliability of the food table in evaluating of calcium was validated with chemical analysis (see Appendix III).

4.4 Measurement of Bone Mineral Mass by Single Photon Absorptiometry (SPA)

The use of Single Photon Absorptiometry in determining bone mineral mass in human subjects has been consented by the Ethical Committee of the Faculty of Medicine, The Chinese University of Hong Kong.

4.4.1 The Instrument

Norland Digital Bone Densitometer, model 2780 (Norland corporation, Fort Atkinson, Wisconsin, U.S.A.). was used to measure bone mineral content (BMC) and bone width (BW) and the ratio of Bone Mineral Content to Bone Width.

The Norland model 2780 densitometer comprises 5 major components: (a) a sealed source of Iodine 125 radionuclide; (b) a scintillation detector with adjustable collimators; (c) a built-in microcomputer with a panel of pre-scan set-up parameters; (d) a video display screen; and (e) an output device for storing pre-scan information and scan data either in computer diskette or print out paper.

The radioactivity of a new Iodine 125 source used in the study was 200 millicurie, its activity decays with time and therefore the source had to be replaced in every 6 months as recommended by the manufacturer. The Iodine 125 source emits a low energy monoenergetic photon beam (27.4 KeV) which is highly collimated to a 3.2 mm band.

The collimated scintillation detector counts the attenuated photon after penetrating through the bone matrix and the surrounding soft tissue. The purpose of photon beam collimation is to limit the reception of the beam to a narrow diameter so that only the photon penetrates vertically upwards through the defined bone region is counted. This is achieved by screwing a tube like metal collimator into the scanner's head. There are three sizes of collimators according to the length of the diameter of the collimator: size 4 (3.2 mm), size 3 (2.4 mm) and size 2 (1.6 mm). The choice of collimator depends on the level of activity remained in the radionuclide source which can be reflected on the photon count rates in pre-scan peak setting. The instrument functions effectively within a range of workable photon count rates. Therefore, in the present study, when the baseline photon counts rates exceeded the recommended 10,000 counts per second, this happened with a newly installed Iodine 125 source. To limit the count rates to a workable level, it was necessary to use a smaller collimator (number 3 or number 2). On the other hand, if the count rates fell below 2,000 counts per second, this occurred when the activity of the radionuclide source was near to an end. It was required to use collimator (number 4) to bring up the count rate to an acceptable level.

The microcomputer controls the synchronizing motions of the scanner and the radionuclide source, it counts the attenuated photon and analyses the scan data to give quantitative end results of BMC, BW and BMD. A curve of beam attenuation representing the bone profile during the course of scanning is displayed on video screen.

4.4.2 Calibration of the Instrument

Routine calibration is required to maintain accuracy and linear response of the instrument. The instrument is calibrated with a standard phantom which was calibrated to Norland's primary standards, and in turn whose values of BMC and BW had been standardised .pa with ashing bones. Calibration was performed once every two weeks as recommended by the manufacturer.

4.4.3 Subject Positioning

In order to obtain accurate and precise results, the movement of the forearm during scanning was allowed as little as possible. A number of precautions have been followed to produce accurate and reliable results.

The child was assured that the measurement was non-invasive, and all he/she had to do was to put the right forearm flat on top of the scanning deck and keep the whole arm steady for a few minutes. The child was seated comfortably in supine position in front of the instrument. The arm movement or fidgeting was restricted. And the child's attention was drawn to the motioned display of the bone profile on the video screen. Gesturing or talking were not allowed.

(a) Locating the measurement site

The measurement site was located at the distal one-third distance between the mid-point of the styloid process of the ulna and the tip of the olecranon of the right arm. The distance between the styloid process and the tip of the olecranon was measured with a tape measure. The distal one-third site was located by dividing the measured distance by 3.

(b) Fastening Forearm Position

The forearm was secured from movement by using the supplied forearm positioning accessories. These consist of a base tray with two inserted centimeter scales, a movable wrist block, a movable elbow stop block, a plastic limb holder bar and two elastic straps.

The tip of the wrist block on the base tray was adjusted by sliding along the centimeter scale to a point equals the distal one-third length of the radius, placed the fully extended right forearm bent at the elbow onto the base tray. The edge of the mid-point of the styloid process was made touching the tip of the wrist block. The forearm was at right angle with the inserted track of the beam path on the base tray. Thus, the cross-section of the measuring site was exactly located perpendicular to the beam path. This position was essential for proper and accurate measurement.

Then, the site was surrounded with a tissue equivalent bag (a rubber bag filled with distilled water) to ensure uniform photon absorption above and below the arm. The bag was positioned so that no gaps or wringles was present between the arm and the bag. Then a limb holder was pushed down firmly with sufficient pressure to hold the arm steady on the scanning deck. Then, fastened the elastic strap by stretching it first and then pulling around the wrist, checked that the styloid process was still touching the tip of the wrist block and the elbow was bent at right angle. Then, slided the elbow stop-block and pushing it against the elbow and lock the position, and then fastened the position of the elbow with another strap.

4.4.4 Setting Up Pre-scan Parameters

It was necessary to warm up the instrument for at least five minutes before measurement. Prior to scanning, it was necessary to set up pre-scan parameters.

(1) Peak Setting

The microcomputer selected the peak energy level (the highest photon counts per second) from the beam profile for scanning purpose. The peak energy was proportional to the activity of the Iodine 125 source. As the activity of the source decayed with time, the peak energy level also declined accordingly. Therefore, peak setting was required each day to set the optimal energy level for bone measurement. The choice of collimator was also dependent on the photon count rates as mentioned in section 4.4.1.

(2) Multiple scanning

This mode instructed the instrument to perform the required number of scans. In the present study, measurement was done in duplicate.

(3) Horizontal scale

This mode set the total width of the displayed region for a bone profile. The region would appear in the detailed measure scan. In the present study, a 4 cm horizontal scale was used for children at five.

(4) Which bone

This mode selected the position of the desired bone relative to the home position of the scanner and the source. In the present study "number 2" was set for the computer to search for the radius.

(5) Threshold

The search threshold differentiated bone edge from soft tissue. The microcomputer set the baseline count rate at 100%, and identified the soft tissue and bone material with photon count rates below this level. 80 to 85% of threshold levels were used in the present study to search for the bone edge.

In summary, the typical pre-scan set-up parameters prior to scanning in the present study are given as follows :

* Peak setting : say, 8,000 counts per second as determined by the radioactivity of the radionuclide
* Multiple Scanning : 2
* Horizontal scale : 4 cm
* Which bone : 2
* Threshold : 85%
* Collimator : 3

4.4.5 Bone Scanning : Search Scan and Measure Scan

After setting the pre-scan parameters, the bone scan commenced. Each complete bone scan consisted of a set of two scans: a search scan which searched for and located the desired bone, in the study it was the radius to be located. And followed by a measure scan which performed the actual determinations of BMC and BW of the bone.

As the scanning started, the shutter of the Iodine 125 source opened, the scanner detector above the arm and the source emitting photon beam under the scanning deck advanced simultaneously to scan the forearm. The scanner would proceed to do a detailed bone profile of the radius (the measure scan). The microcomputer would calculate the BMC, BW, and the ratio of BMC/BW. The scan results together with the pre-scan parameters and the bone profile would be displayed on the screen. Having computed the measurement in duplicate the microcomputer would calculate the mean values and standard deviations of BMC, BW and BMC/BW of the two scans. The results were displayed on the screen and recorded in print out form.

4.4.6 Evaluation of the Accuracy and Precision of Bone Mineral Content Measurement

4.4.6.1 Accuracy and Precision of the Single Photon Densitometer

The accuracy of the densitometer in measuring BMC and BW was evaluated by a series of 11 multiple measurements (3 - 5 scans each) of the four chambered standard bone phantom over a six month period.

The precision of the densitometer in measuring BMC and BW was determined by a series of multiple measurements on the bone phantom over the six months period. The precision measurement was sub-divided into short term (daily) and long term (once a fortnight) precision measurements. The precision was determined by calculating the coefficients of variation of multiple measurements (five scans each) of the bone phantom.

The values of BMC and BW measured by the densitometer were highly correlated with the true values of the bone phantom (r = 0.999) for both BMC and BW. The coefficients of variation for multiple measurements of bone phantom were 0.3% to 1.71% for BMC and 0.12% to 1.32% for BW. Therefore the instrument is accurate and precise to measure BMC and BW in vivo.

4.4.6.2 Evaluation of the Operator's Technique in Performing BMC and BW Measurements

The immediate reproducibility, that is the precision of measurement from scan to scan without repositioning of the subject. In this study, the measurements of BMC and BW were obtained from an average of two scans. The immediate reproducibility was determined by the average of the coefficients of variation between scans of all the measurement.

The reproducibility of the measurement after repositioning of the subject for scan was performed in 12 randomly selected children. The interval between two measurements was within the same day.

The average coefficient of variation in two successive scans without repositioning were 2.49% and 2.03% for BMC and BW respectively. This high degree of precision was achieved requiring an excellent cooperation of the children and comfortable positioning in order to keep the children comfortable and steady during scanning. The intra-class correlations between measurements with repositioning of the forearm were 0.937 for BMC and 0.906 for BW. Therefore, the technique in locating the forearm for measurement was highly reliable.

4.4.6.3 Comparison of the Difference in BMC between Dominant and Non-Dominant Arm

The right forearms of the studied children were exclusively used for BMC measurement. In order to find out if there is any significant difference in BMC between the dominant and non-dominant forearm of children at five years of age. A sub-group of 27 children were selected randomly to have their both forearms measured at the distal one-third site. The significance of the result was statistically analysed by paired t-test . The use of paired t-test shows that there is no significant difference in BMC between the dominant and non-dominant arm in children at five years old ($t=1.596$, d.f.$=26$, $p>0.5$).

4.4.7 Radiation Exposure

The Iodine 125 radionuclide is a weak radioactive source, the scanning of the forearms is localised at a narrow cross section of the radius. The photon beam is highly collimated to about 4.5 mm in diameter at the measuring deck and at the detector is about 4 mm in diameter. The beam is completely absorbed by the detector. The average maximum radiation dose of the subject was calculated to be about 234 mR for every measurement (two scans in each measurement) for a 200 millicurie and decreasing proportionally with the age of the source.

Chapter 5

Results

5.1 Sample Size

Between June to December 1989, 133 five years old cohort children (70 boys and 63 girls) returned for follow-up study. Dietary assessment records were obtained in a 40 minutes interview for each child. The parents were very cooperative and were patient throughout the interview.

Six out of 133 children refused to have their forearms measured by the single photon densitometer because they were scared of being fastened to the instrument. Hence only 128 records of bone mineral measurements were obtained.

5.2 Representative of the Sample

To review the representative of the samples, the father's occupations were classified according to the Quarterly Report of The General Household Survey (Hong Kong Government Census and Statistics Department 1990) in order to compare with the distribution of occupations in the average population. (see Table 2). In the category of "Production and related workers, ...", approximately 10% (the difference of the two percentages) over-representation was found in the study families in comparison with the corresponding percentage in the average population (see Table 2). Otherwise, the differences in percentage in the rest categories were less than 10%. Therefore, this group of children should well represent the socio-economic classes in Hong Kong.

5.3 Weight and Height

The mean (SD) weights and heights of 133 Hong Kong Chinese children are shown in Table 3.

5.4 Validity of the Food Composition table

A 7-day's food collection was used in validating the compiled food composition table. The calcium content calculated from the food table was compared with those obtained by the method of chemical analysis. The results are given in Table 4. The value of food calcium as determined by the food composition table was well correlated with the value obtained by chemical analysis ($r=0.8$). The average percentage difference of calcium content analysed by the composition food table was 15.8% less than the method of chemical analysis. Therefore, the compiled food composition table is reasonably accurate and reliable in determining calcium content in food (Appendix III).

5.5 Calcium Intake of Children at Five

The mean daily intakes of calcium, energy and protein of the children are shown in Table 5. The mean daily intake of calcium in Hong Kong Chinese children at five years of age is comparable with the FAO/WHO (1962) recommendation of calcium intake (400 - 500 mg/day), and also achieves two-thirds of the U.S. recommendation (800 mg/day) (National Research Council 1989).

The chief source of dietary calcium in these children was derived from milk and milk products (43.5%) (see figure 2). Cereals and vegetables were the next two important sources of calcium, each contributed respectively 16.6% and 12.5% of total dietary calcium.

Over 90% of these children maintained milk drinking habit up to five years of age. Most of these children took fresh milk, full cream powdered milk. Some of them have been using follow-on formulae since weaning. Cheese and yoghurt were less popular among the children. The level of calcium intake is found to be proportional to the frequency and quantity of the consumption of milk. Our children consumed little bean and bean products such as baked bean, dried bean curd sticks and tofu, etc. Therefore, the contribution of calcium from this food group was less than 2%.

Figure 3 shows the distribution of calcium intake in the studied children. There are 25 children with mean calcium intake below 300 mg/day. The dietary sources of calcium in these children were mainly derived from non-dairy food namely cereals, vegetables and fruits. Without a regular consumption of milk a few number of these children could take in more than 300 mg of calcium per day .

Fifteen children had calcium intake below 250 mg/day. Three of them in average took less than 200 ml of milk a week. The rest of the children stopped using milk at five years of age.

5.6 Bone Mineral Content (BMC), Bone Mineral Density (BMD) and Bone Width (BW) of 128 Hong Kong Children at Five

The mean BMC, BMD and BW of the children at five is shown in Table 6. There is no sex difference between BMC and BMD using two samples t-test. However, there is a significant sex difference in BW ($t = 3.53$, d.f. = 126, $p < 0.001$).

The BMC in these 128 children are correlated with weight, height, BMD, and BW (see Table 7). These findings indicate that body weight and height have profound effects on predicting BMC. However, BMC does not correlate with current energy intake ($r=0.13$, $p=0.13$) and current protein intake ($r=0.04$, $p=0.66$). The most interesting finding is that there is no relation between BMC and current calcium intake at age five ($r=0.13$, $p=0.14$). In other words, the BMC would lie within a narrow range between 0.23 to 0.44 g/cm at a wide range of calcium intakes from about 170mg/day to 1400 mg/day (see Figure 4).

5.7 Comparisons of Bone Mineral Content (BMC) in Children with High and Low Calcium Intake

In order to see whether children with low calcium intakes ($\leq$ 250mg/day) had lower BMC than the rest of the children. The children were divided into three groups according to calcium intake at five: Low intake (calcium intake $\leq$ 250 mg/day, BMC = 0.3083 mg/day, n = 15); medium intake (250mg < calcium intake $\leq$ 900mg/day, BMC = 0.3167 g/cm, n = 103) and high intake (calcium intake > 900 mg/day, BMC = 0.3297g/cm, n = 15). The differences in BMC between the low intake and the other two groups are, however, non-significant; when BMC was regressed against two dummy variables representing the medium and high intake groups using the low intake groups as the reference (p = 0.2711 and 0.1689 respectively, R^2 = 0.015). Such differences also remain non significant after adjusting for sex, weight, height and bone-width. Therefore, the statistical analysis indicates that a higher calcium intake at age five is not associated with higher BMC in Hong Kong Chinese Children and vice versa.

Actually, the bone of children at five should reflect calcium that has been deposited over the past years, it would be more appropriate to study cumulative calcium intake from birth to five years. Records of dietary calcium intake from birth to four years on 15 occasions - two monthly in the first year, three monthly in the second year and third year, then yearly in the fourth and fifth year were used to estimate the total calcium intake and average daily calcium intake in every child. The total calcium intake from birth to five years could be estimated by the area under the polygon formed by mean daily calcium intakes from the 16 dietary assessments, with x-axis in days.

The average calcium intake per day in the five years period can be estimated by dividing the total calcium intake by the total number of days (a constant factor), both measures are going to give exactly the same p value and partial correlation. The latter is used in the following analysis because we are more used to the magnitude of daily intake.

The correlation coefficient of average daily calcium intake over the first five years is found to be significantly correlated with BMC at age five ($r = 0.235$, $p = 0.0133$) (Figure 5). It seems that children with higher calcium intake over the first five years of life have higher BMC value.

In order to determine whether calcium intake at a particular stage of life was more significantly associated with BMC, a statistical approach termed "Principle Component Analysis" was suitable for this purpose. The previous 15 dietary assessments for calcium from birth to age five (the first dietary assessment was excluded for its large number of missing values) were reduced into four factors which were orthogonal or not correlated with each other. The 15 variables loaded distinctively onto the four factors mentioned, which totally took up 71.8% of the variance. Such four sets of factors scores could be interpreted as calcium intake from the second to 4th month, 6th to 10th month, 15th to 24th month and 27th to 60th month. When the four factors were used to predict BMC, **calcium intake from 15th to 24th month is significantly associated with BMC (r=0.240, p=0.02).** While calcium intakes in the other months are non-significant. The association remains significant after adjusting for weight, height, bone width and sex (partial r=0.237, p=0.02). **The result suggests that calcium intake in the second year of life has a significant prediction on BMC at five year.**

A further analysis was required to see whether the significant correlation between the average daily calcium intake over the first five years and BMC could be explained by other factors such as weight, height, sex, bone width or calorie intake. This was analysed by the multiple regression model. The average daily calcium intake over five years remains significant (partial r = 0.248, p=0.0107) after controlling for other variables mentioned. Body weight and bone width have independent effects to predict BMC. On the other hand, height, sex and current energy intake are non-significant (see Table 8). It is interesting to see that the overall model is highly significant and explains for 55% of the variance of BMC.

If the body mass index (wt/ht^2) was used instead of weight, a similar model could be obtained (R^2 = 54%) but height now becomes significant (partial r = 0.321, p = 0.0008). Whether the use of weight or body mass index does not seem to make much difference in predicting BMC.

Attempt has been made to divide the average daily calcium intake in the first five years into three groups.

Higher intake if calcium intake was greater than 750 mg/day, medium intake if calcium intake was between 400 to 750 mg/day and low intake if below 400 mg/day. Both the high calcium intake group and the medium calcium intake group have significantly higher BMC than the low intake group. ($p < 0.05$)

In a previous section, it was found that the current intakes of calcium and protein are not correlated with BMC. However, the average calcium intake over the first five years is significantly correlated with BMC at five. It would be interesting to examine whether the average intakes of protein and energy over the first five years are independent predictors on BMC at five because intakes of energy and protein over a prolonged period of time may have important effects on bone development. The approach in estimating average protein and energy intakes over the first five years was similar to the one in estimating average calcium intake over the first five years. Table 9 presents the results of a multiple regression analysis examining the independent effects of average intakes of protein and energy over the past five years on BMC by controlling confounding factors, namely average calcium intake over the first five years, weight, height, bone width and sex. The results show that the cumulative intakes of protein (partial $r = 0.014$, $p = 0.8867$) and energy (partial $r = 0.061$, $p = 0.5368$) do not correlated with BMC after controlling the variables mentioned. Therefore, the cumulative intakes of protein and energy in the past five years were not significant predictors on BMC at five. Whereas the average calcium over the first five years , weight and bone width at five years remain significant in predicting BMC at five. The overall regression model is highly significant and explains for 54% of the variance of BMC.

In conclusion, the results are consistent to suggest that higher cumulative calcium intake being significantly correlated with higher BMC in the first five years of life, though no association was apparent for calcium intake at age five. Calcium intake, particularly in the second year of life was the most significant predictor on BMC at five years of age. Cumulative intakes of protein and energy during the first five years of life do not appear to have significant influence on BMC at age five. On the other hand, body weight, height, body mass index and bone width are important variables in predicting BMC.

5.8 Relationships between Bone Mineral Density (BMD), Body Size and Dietary Intakes of Calcium, Protein and Energy

Although Bone mineral density (BMD) is not a proven valid bone variable to express the quantity of bone mass as discussed in a section 3.3.1.2, it would be worthwhile to try examining the relationships of BMD with anthropometric variables and dietary intakes.

The BMD of the children is significantly correlated with weight ($p<0.001$), height ($p=0.001$) and current calcium intake ($p=0.029$) (see Table 10 and Figure 6). These findings suggest that body weight, height and calcium intake at age five are significant factors influencing BMD. However, energy and protein intakes at age five are not significantly associated with BMD.

An attempt was made to examine whether body weight, height, sex, current dietary intakes of calcium, protein and energy would have independent effects on predicting BMD. A multiple regression analysis was performed. Calcium intake at five, as an independent variable, was found to be more significantly correlated with BMD after controlling for the mentioned variables (partial $r = 0.241$, $p=0.0072$) (see Table 11). Body weight remains a significant factor to predict BMD. No significant correlations were noted in the model between BMD and height, sex, protein and energy intakes at age five. The overall model, however, is not significant and explains for only 20% of the variance of BMD.

Therefore, the statistical analysis indicates that a higher calcium intake at age five is significantly associated with higher BMD in Hong Kong Chinese children.

In a previous section it was noted that cumulative calcium intake over the past five years is significantly correlated with BMC whereas dietary variables such as cumulative intakes of protein and energy do not demonstrate any significant relationships with BMC. In order to examine whether such dietary variables have similar relationships with BMD, a correlation analysis was performed to relate cumulative calcium intake in the past five years with BMD. Figure 7 shows the relationship between BMD of the children and their cumulative calcium intake over the first five years. The BMD of the 128 Hong Kong Chinese children is significantly correlated with their cumulative calcium intake ($r=0.2017$, $p=0.035$).

Furthermore, a multiple regression analysis was carried out to examine whether the cumulative calcium intake is an independent variable on predicting BMD. After adjusting for variables such as weight, height, sex, cumulative intakes of protein and energy, the cumulative calcium intake remains significantly correlated with BMD (partial r = 0.203, p=0.0381) (see Table 12). Body weight remains significant in predicting BMD. No significant correlations have been noted between BMD and height, sex, cumulative intakes of protein and energy of the past five years. The overall model is still non-significant and explains for only 20% of the variance of BMD. This may suggest that some unidentified variables being significant predictors on BMD have not been entered into the regression models.

5.9 Inter-correlations between Bone Mass Measurements, Body Size and Dietary Intakes in Hong Kong Children

A correlation matrix between the variables of bone mass measurements, body size and dietary intakes in Hong Kong children are shown in Figure 8. There is a high correlation between weight and height (r=0.7563, p<0.0001). Bone width is also highly correlated with weight (r=0.3971, p<0.0001) and height (r=0.3302, p<0.0001). BMC is highly associated with bone width (r=0.6631, p<0.0001). Height has a significant correlation with energy intake at five years (r=0.1935, p=0.026) and cumulative energy intake (r=0.1892, p=0.035). Weight is not associated with cumulative energy intake (r=0.1212, p=0.180). However, weight is significantly related to current energy intake (r=0.2621,

$p < 0.002$). Calcium intake is highly correlated with protein intake ($r=0.4631$, $p<0.0001$), energy intake ($r=0.5386$, $p<0.0001$) and cumulative calcium intake ($r=0.6374$, $p<0.0001$). As the variables of bone mass, body size , dietary intakes of calcium, energy and protein are inter-correlated, it is necessary to use multiple regression analysis to examine the independent effect of each of the variables on BMC or BMD.

5.10 Planning for Further Investigation

Would the association between BMC and calcium intakes be consistent in a large group of children who have a habitual low calcium diet? Thus, a further investigation was performed to study the bone mineral content of a group of children who had continuously low calcium intake. The details of the investigation is presented in the following chapter.

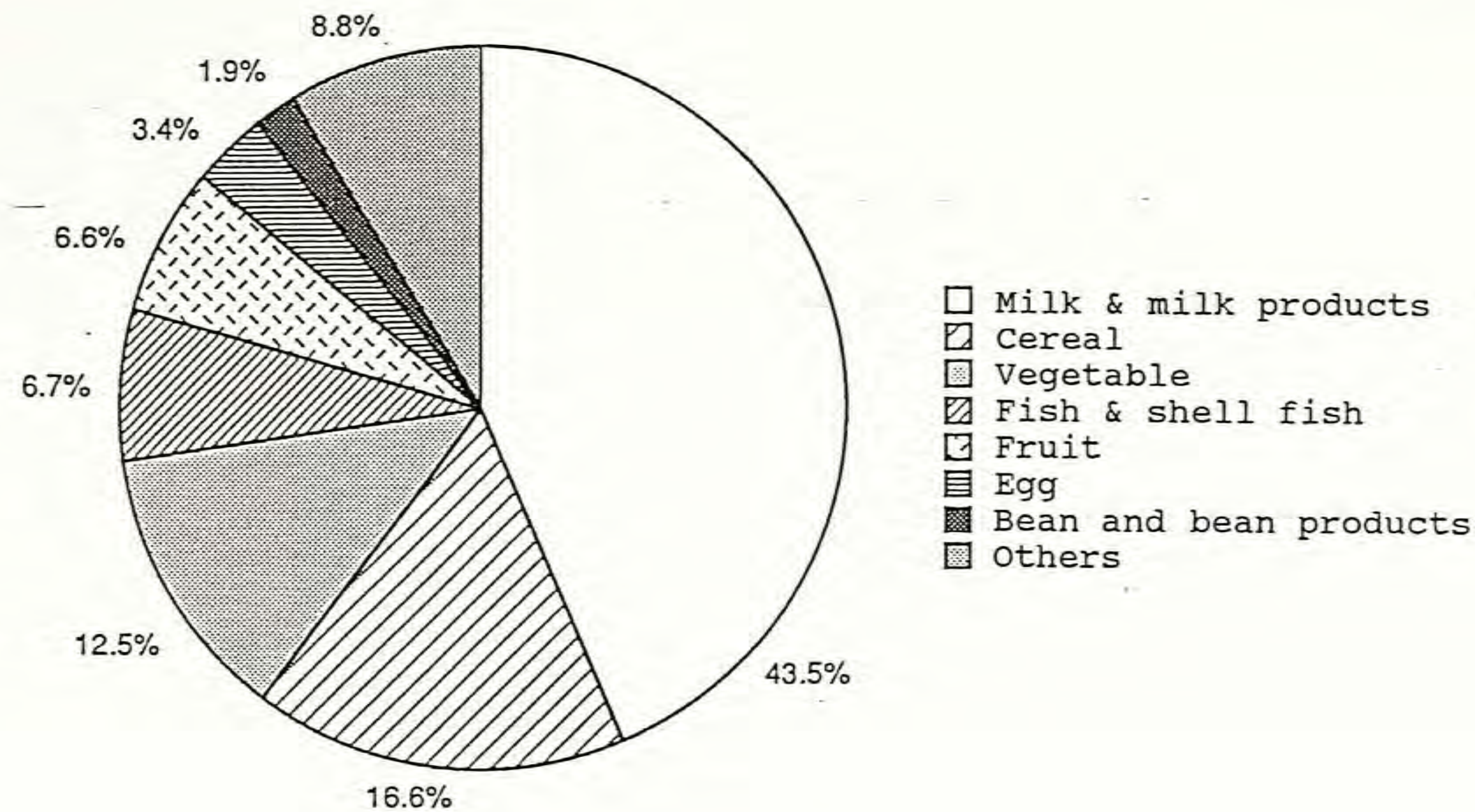

Figure 2 Sources of dietary calcium in Hong Kong Chinese children at five years old

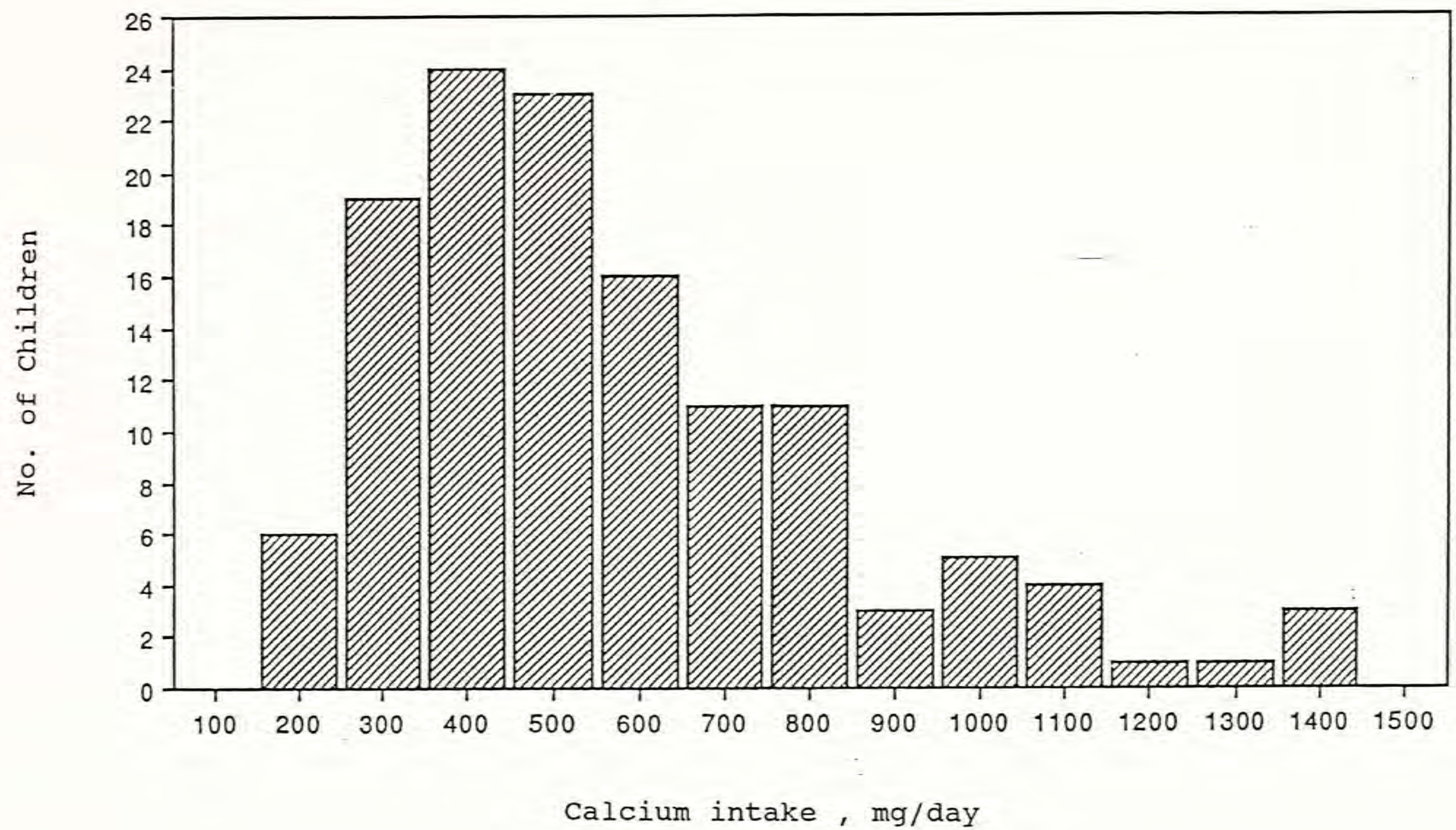

Figure 3 Distribution of calcium intake in *127 Hong Kong Chinese children

* A boy with calcium intake above 2,600 mg/day was not shown in the figure

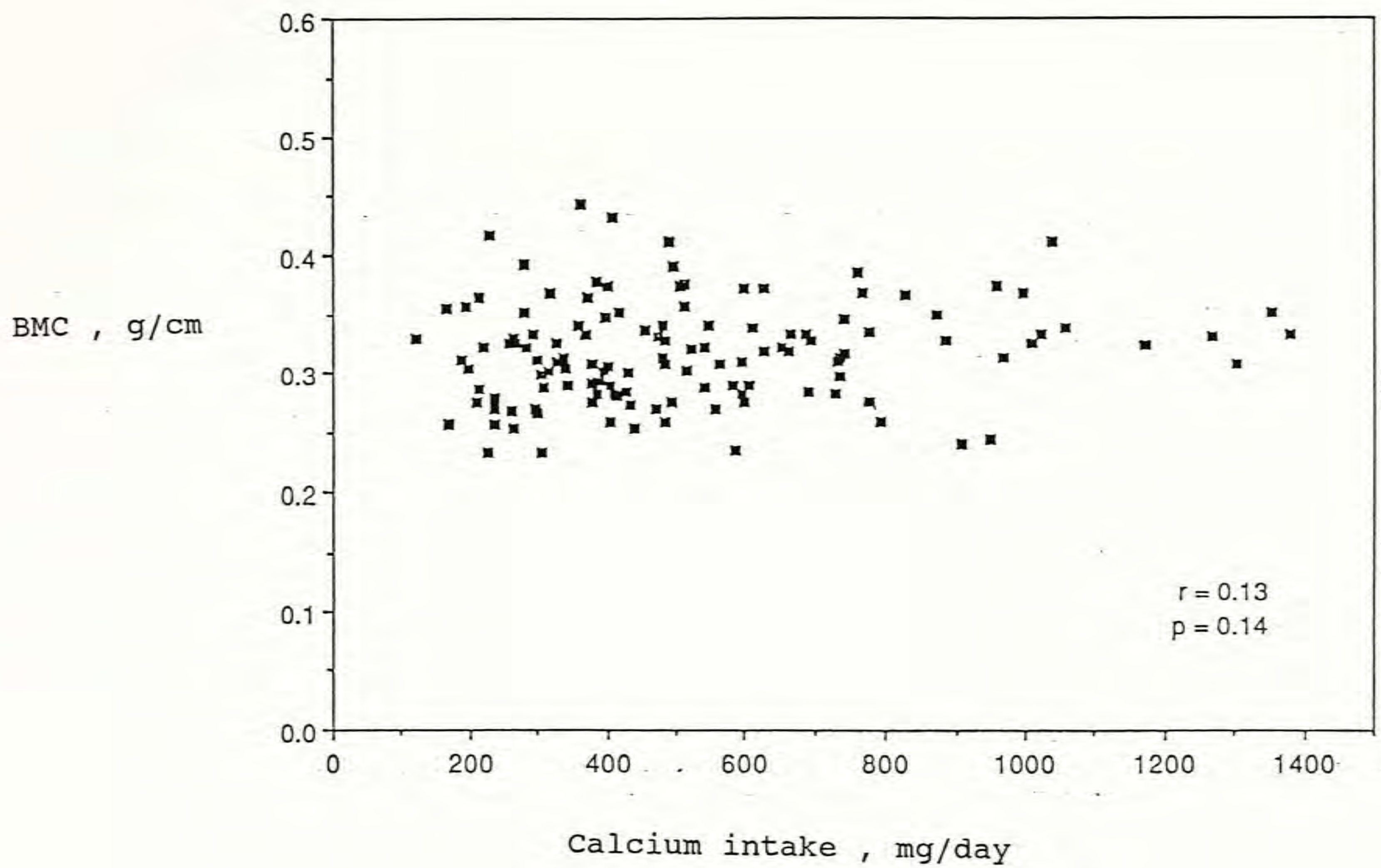

Figure 4 Correlation of bone mineral content (BMC) and calcium intake in *127 Hong Kong Chinese children at five years old

* A boy with calcium intake above 2,600 mg/day & BMC of 0.342 g/cm is not shown in the figure.

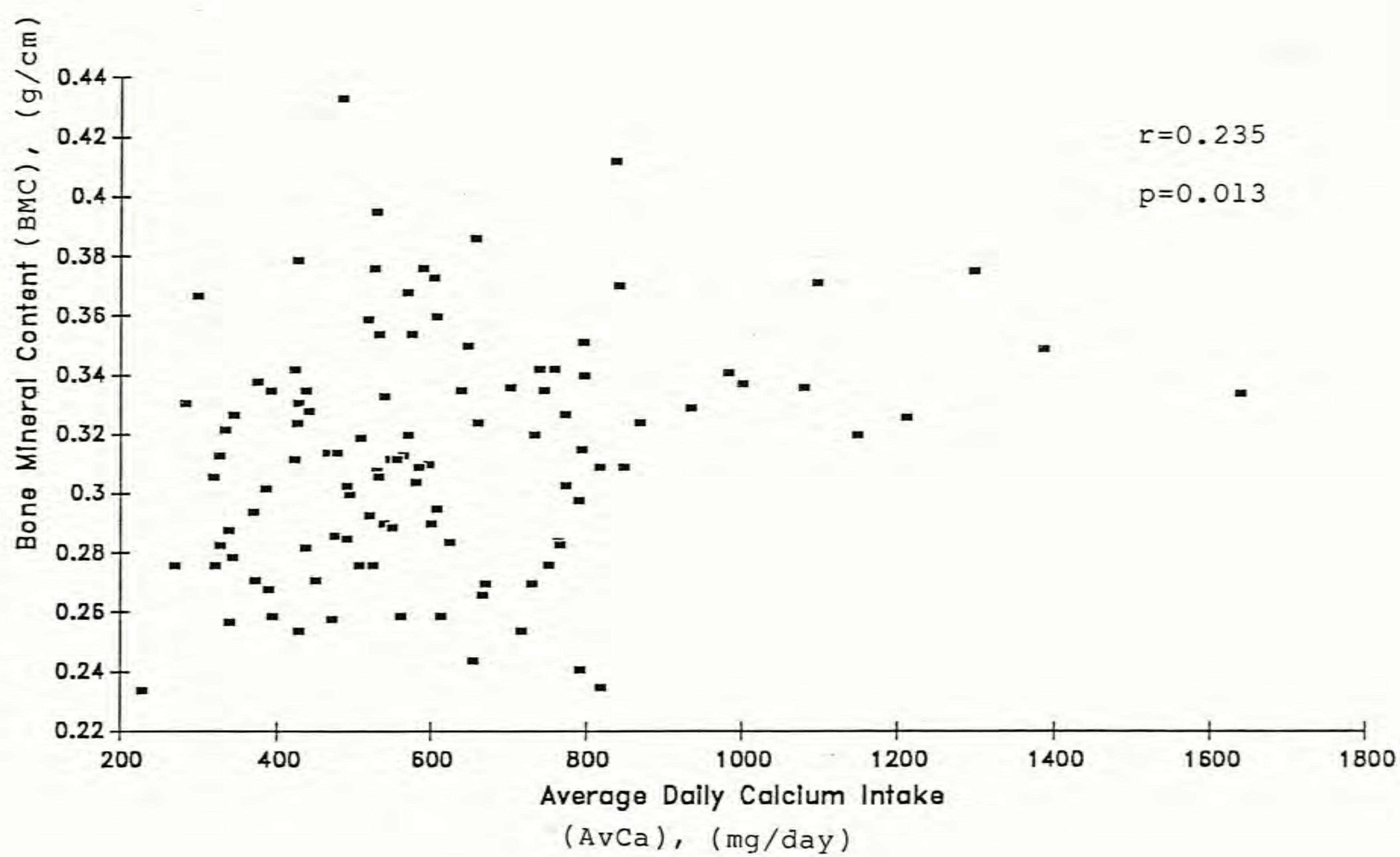

Figure 5 Correlation of bone mineral content (BMC) and average calcium intake over the past five years (AvCa) in 128 Hong Kong Chinese children

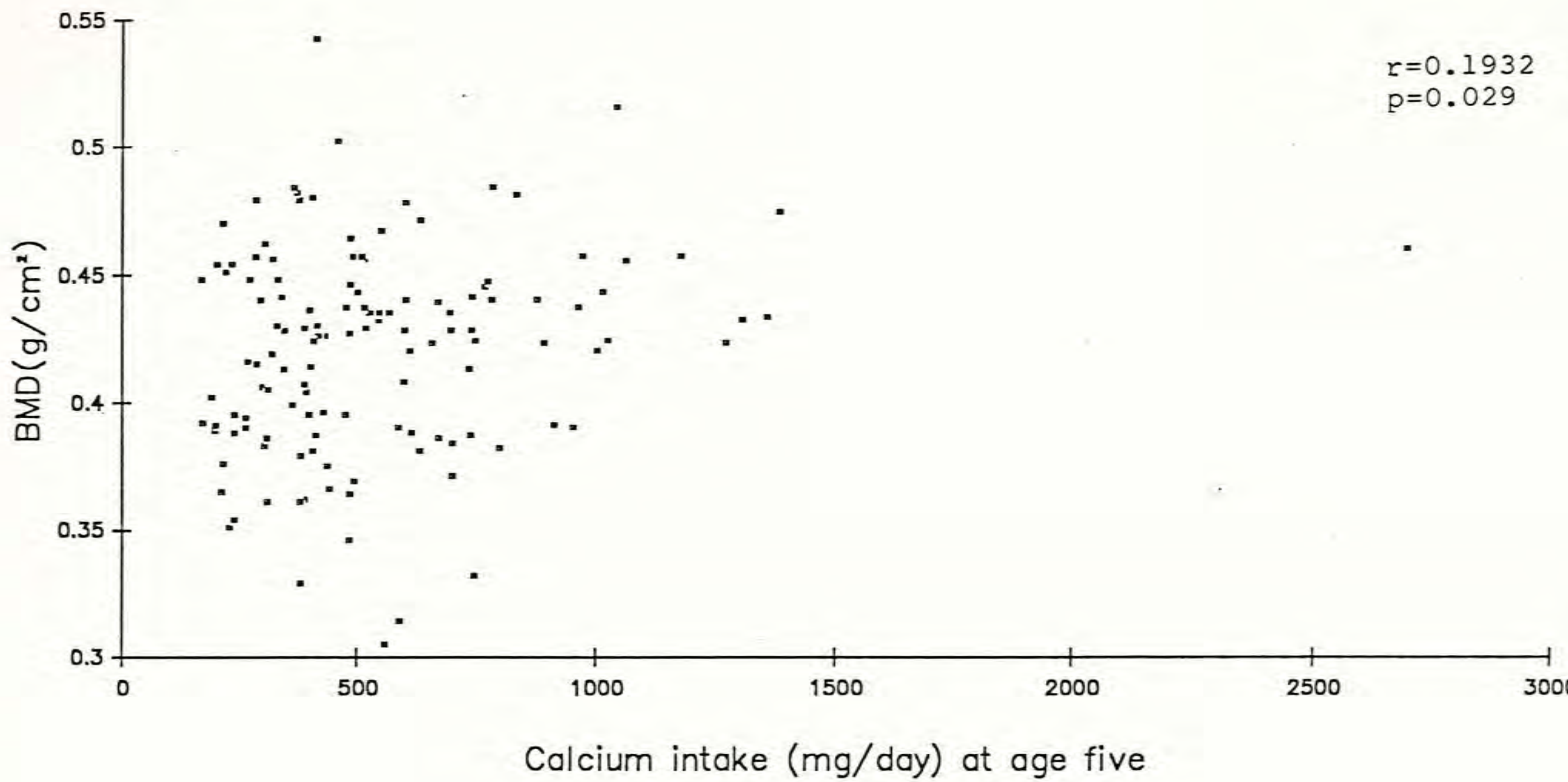

Figure 6 Correlation of bone mineral density (BMD) and calcium intake at age five in 128 Hong Kong Chinese children

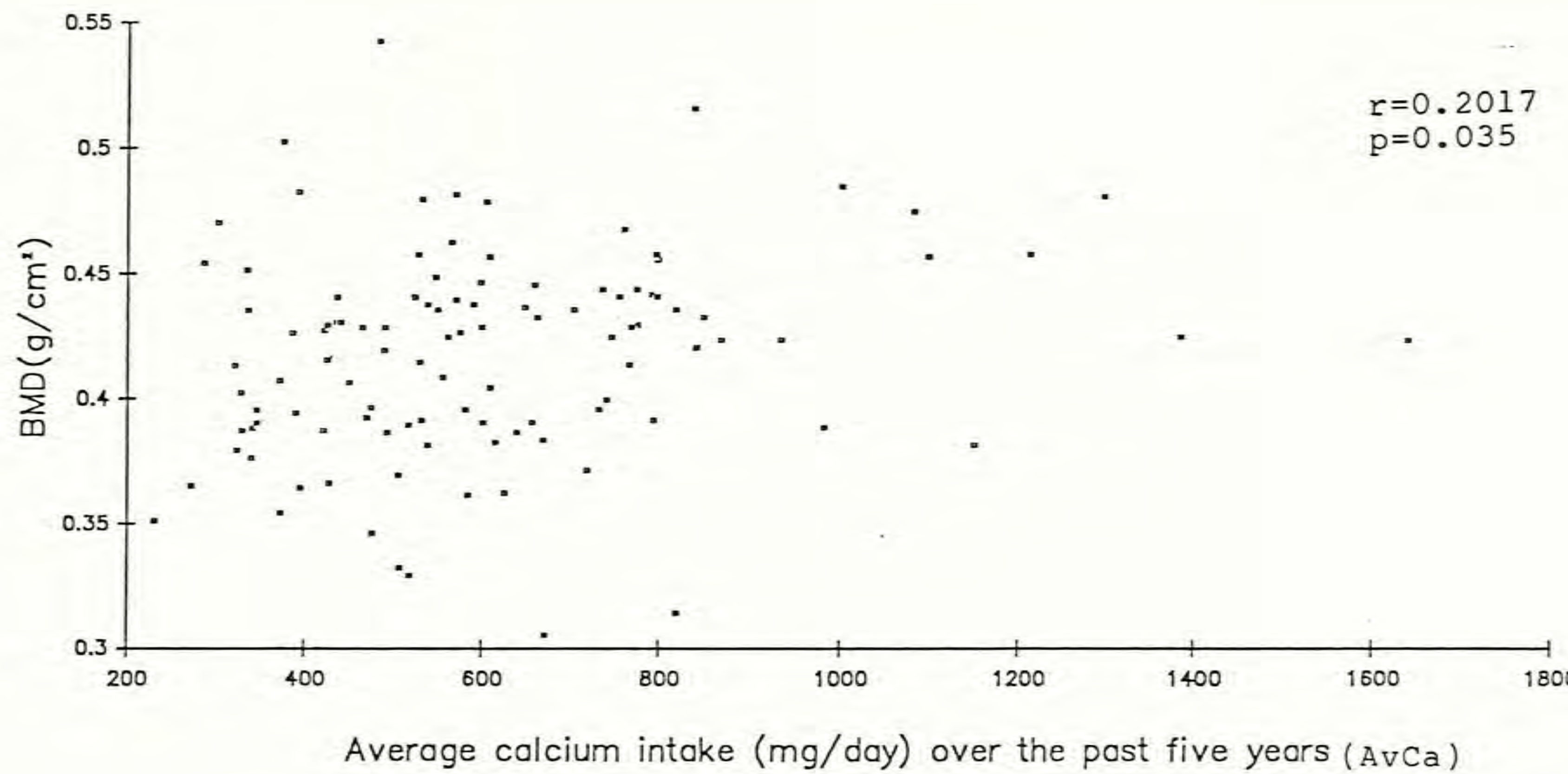

Figure 7 Correlation of bone mineral density (BMD) and average calcium intake over the past five years (AvCa) in 128 Hong Kong Chinese children

		BMC	BMD	AvCa	Height	Weight	BW	Sex	AvProt	Protein	AvKcal	Calcium	Energy
BMC	r=	-	0.6959	0.2355	0.4675	0.5698	0.6631	0.1676	0.0437	0.0397	0.1610	0.1312	0.1321
	p=	-	0.0000	0.013	0.0000	0.0000	0.0000	0.059	0.624	0.656	0.079	0.140	0.137
BMD	r=			0.2017	0.2964	0.3652	0.0696	0.0482	0.0266	0.0420	0.0808	0.1932	0.0052
	p=			0.035	0.001	0.0000	0.435	0.589	0.766	0.637	0.380	0.029	0.953
AvCa	r=				0.1403	0.1032	0.0992	0.375	0.5990	0.2517	0.6477	0.6374	0.2806
	p=				0.140	0.279	0.302	0.693	0.0000	0.007	0.0000	0.0000	0.003
Height	r=					0.7563	0.3302	0.0458	0.1079	0.1473	0.1892	0.0835	0.1935
	p=					0.0000	0.0000	0.602	0.218	0.092	0.035	0.341	0.026
Weight	r=						0.3971	0.1150	0.0483	0.1750	0.1212	0.1542	0.2621
	p=						0.0000	0.189	0.582	0.045	0.180	0.077	0.002
BW	r=							0.2995	0.0680	0.0078	0.1430	0.0084	0.2003
	p=							0.001	0.446	0.930	0.126	0.925	0.023
Sex	r=								0.0405	0.1227	0.1126	0.0927	0.2781
	p=								0.643	0.159	0.211	0.289	0.001
AvProt	r=									0.3555	0.8790	0.1407	0.3374
	p=									0.0000	0.0000	0.106	0.0000
Protein	r=										0.3980	0.4631	0.7934
	p=										0.0000	0.0000	0.0000
AvKcal	r=											0.3206	0.4863
	p=											0.0000	0.0000
Calcium	r=												0.5386
	p=												0.0000
Energy	r=												-
	p=												-

Figure 8 Correlation coefficients (r) between the variables of bone mineral content (BMC), bone mineral density (BMD), height, weight, bone width (BW), sex, intakes of protein, calcium and energy at five years of age; average intakes of calcium (AvCa), protein (AvProt) and Energy (AvKcal) over the past five years.

Table 2

Distribution of fathers'occupations of the 133 Hong Kong Chinese childern in comparison with *Hong Kong household survey in June 89

Occupation	This Study		*June 89
	Number	%	%
Professional, administrative and managerial workers	22	(16.5)	11.3
Clerical and related workers	16	(12.0)	18.7
Sales workers	7	(5.3)	12.3
Service workers	21	(15.8)	16.9
Production and related workers, transport equipment operators and lablurers	67	(50.4)	39.5
Others	0	(0)	1.3
Total	133	(100)	100.00

* Hong Kong Government Census and Statistics Department (1990).

Table 3

Mean weight and height of 133 Hong Kong Chinese children at five years old

	No.	Weight(kg) Mean (SD) [Range]	Height(cm) Mean (SD) [Range]
Boys	70	17.6 (2.3) [13.6 - 27.8]	108.2 (4.0) [96.8 - 118.6]
Girls	63	17.1 (2.6) [13.0 -27.9]	107.9 (4.3) [99.3 - 117.3]
Total	133	17.4 (2.5) [13.0 - 27.9]	108.1 (4.18) [96.8 - 118.6]

Table 4

Total calcium content in each of the 7-day's food collection as determined by the compiled food table and chemical analysis

Day	Compiled Food Table (mg/d)	Chemical Analysis (mg/d)
1	367.2	441.8
2	454.4	385.2
3	276.7	325.0
4	415.6	436.2
5	204.6	175.3
6	260.9	312.4
7	301.7	392.7

Table 5

Mean daily intakes of calcium, energy and protein of 133 five years old Hong Kong Chinese children

	No.	Calcium (mg/day) Mean (SD) [Range]	Energy (Kcal/day) Mean (SD) [Range]	Protein (g/day) Mean (SD) [Range]
Boys	70	574.1 (351.9) [170.1 - 2689.1]	1489 (316) [865 - 2446]	68.9 (17.3) [33.6 - 98.9]
Girls	63	514.1 (290.6) [166.8 - 1378.8]	1325 (254) [792 - 2055]	65.2 (16.3) [32.3 - 96.1]

Table 6

Mean bone mineral content (BMC), bone mineral density (BMD) and bone width (BW) of 128 Hong Kong Chinese children at five years old

		BMC (g/cm)	BMD (g/cm^2)	BW (cm)
	No.	Mean (SD) [Range]	Mean (SD) [Range]	Mean (SD) [Range]
Boys	67	0.324 (0.040) [0.240 - 0.432]	0.418 (0.041) [0.304 - 0.541]	0.777 (0.073) [0.616 - 0.962]
Girls	61	0.310 (0.046) [0.232 - 0.443]	0.422 (0.040) [0.313 - 0.501]	0.733 (0.068) [0.610 - 0.932]
Total	128	0.317 (0.042) [0.232 - 0.443]	0.420 (0.041) [0.304 - 0.541]	0.756 (0.074) [0.610 - 0.962]

Table 7

Correlation coefficients (r) of bone mineral content (BMC) with weight, height, bone mineral density (BMD), bone width (BW), energy intake, calcium intake and protein intake of 128 Hong Kong Chinese children at five years old

	r	* p
Weight (kg)	0.57	< 0.01
Height (cm)	0.47	< 0.01
BMD (g/cm^2)	0.70	< 0.01
Bone width (cm)	0.66	< 0.01
Energy (Kcal/day)	0.13	0.13
Calcium (mg/day)	0.13	0.14
Protein (g/day)	0.04	0.66

* $p < 0.01$ denotes significant.

Table 8

Partial correlation coefficients of Bone mineral content (BMC) with average calcium intake over five years (AvCa), height, weight, bone width (BW), sex and current energy in 128 Hong Kong Chinese children

	Partial Correlation coefficient	P
AvCa (mg/day)	0.248	0.0107
Height (cm)	0.025	0.7193 (NS)
Weight (kg)	0.406	<0.0001
BW (cm)	0.481	<0.0001
Sex	0.035	0.719 (NS)
Energy (kcal)	0.010	0.1337 (NS)

NS denotes not Significant

Table 9

Partial correlation coefficients of bone mineral content (BMC) with weight, height, bone width (BW), sex, average intakes of protein (AvProt), energy (AvKcal) and calcium (AvCa) in 128 Hong Kong Chinese children

	Partial Correlation Coefficient	p
Weight (kg)	0.393	<0.0001
Height (cm)	0.018	0.8568 (NS)
BW (cm)	0.467	<0.0001
Sex	0.058	0.5620 (NS)
AvProt (g/day)	0.014	0.8867 (NS)
AvKcal (Kcal/day)	0.061	0.5368 (NS)
AvCa (mg/day)	0.222	0.0237

NS denotes non-significant

Table 10

Correlation coefficients of bone mineral density (BMD) with weight, height, current intakes of energy, calcium and protein in 128 Hong Kong Chinese children at age five

	r	p
Weight (kg)	0.365	<0.001
Height (cm)	0.296	0.001
Energy (Kcal/day)	0.005	0.953 (NS)
Calcium (mg/day)	0.193	0.029
Protein (g/day)	0.042	0.637 (NS)

NS denotes non-significant

Table 11

Partial correlation coefficients of bone mineral density (BMD) with weight, height, sex and current intakes of calcium, protein and energy in 128 Hong Kong Chinese children at age five

	Partial Correlation Coefficiient	p
Weight (kg)	0.234	0.0091
Height (cm)	0.046	0.6161 (NS)
Sex	0.068	0.4550 (NS)
Calcium (mg/day)	0.241	0.0072
Protein (g/day)	0.089	0.3293 (NS)
Energy (Kcal/day)	0.071	0.4360 (NS)

NS denotes non-significant

Table 12

Partial correlation coefficients of bone mineral density (BMD) with weight, height, sex, average intakes of calcium (AvCa), protein (AvProt) and energy (AvKcal) over five years in 128 Hong Kong Chinese children

	Partial Correlation Coefficient	p
Weight (kg)	0.288	0.0028
Height (cm)	0.022	0.8272 (NS)
Sex	0.158	0.1066 (NS)
AvCa (mg/day)	0.203	0.0381
AvProt (g/day)	0.095	0.3345 (NS)
AvKcal (Kcal/day)	0.136	0.1656 (NS)

NS denotes non-significant

Chapter 6

A Study of Calcium Intake and Bone Mineral Content of Children at Five Years Old in Jiangmen, Guangdong, China

6.1 Introduction

A further investigation of calcium intake in relation to bone mineral content in pre-school children was carried out in Mainland China because it has been repeatedly reported by the Chinese nutritionists that the calcium intake in their pre-school children was low. Two recent dietary surveys conducted in China (Ho ZC, 1988 & (陈钧同寅 1989), (see Table 13) indicated that the average calcium intake in pre-school children between three to five years was around 310 mg/day. The low calcium intake in these children was due mainly to the lack of milk and milk products in the diet since the intake of dairy products is not customary in China.

Jiangmen is a small city in the southern part of Guangdong province in China where children have a similar eating pattern as those of Southern parts of China. With the assistance from the public health doctors at the Department of Public Health, Sun Yat Sen University of Medical Sciences (SYSUMS) in Guangzhou of China, a kindergarten in Jiangmen was approached to carry out a cross-sectional study of bone mineral content and calcium intake in pre-school children. In February, 1989 the same single photon densitometer was shipped to Jiangmen to carry out the study .

6.2 Subjects, Materials and Methods

6.2.1 Subject Selection

A majority of Hong Kong Chinese originated from Guangdong province. To compare children originated from the same province might minimise the effect of racial difference on calcium metabolism.

The studied children came from the second largest kindergarten in Jiangmen run by a light industrial corporation in the city. There are 700 children aged between three to six years. The corporation employs more than 20,000 employees whose pre-school children usually go to this subsidized kindergarten. The kindergarten opens from 7:00 am to 4:00 pm and the children go to the kindergarten six days a week.

In order to recruit sufficient number of children in comparable age with the study group in Hong Kong, Children whose age was between four and a half years and five and a half years from the day of bone mineral measurement were selected. These were normal healthy children and free from any metabolic disease or recent fractures. A total of 115 children (63 boys and 52 girls) were recruited for the measurement of bone mineral content.

6.2.2 Weight and Height Measurements

Weight was measured on a beam balance in under clothes and height was measured without shoes on.

6.2.3 Dietary Assessment

The actual dietary assessment of calcium intake in these children was carried out by the public health doctors in the Department of Public Health, SYSUMS in December, 1989.

Only 16 children in the five years age group were selected to participate in the dietary survey.

Dietary assessment was conducted in five consecutive days from Monday to Friday. Food intake in the kindergarten was assessed by weighed intake method. The methodology has been reviewed in chapter 3. The kindergarten provides breakfast, lunch and afternoon snack everyday. During the study period, all individual food items and beverages of each subject was weighed and recorded immediately before eating. And the procedure was repeated for any food item left over.

Digital electronic scales accurate to one decimal place were used in weighing food. For composite dishes, the cooks from the kindergarten were approached to obtain the information of recipes and cooking method.

Dietary assessment of food intake after school was assessed by food record method. The methodology has been reviewed in Chapter 3. Before the study, the parents were instructed to recognise and describe food portions. Food models and graduated household measures , e.g. cups, spoons, and bowls were on display as an aid to assist description. The doctors also demonstrated to the parents on describing and recording different kinds of food. The parents were asked to practise the recording procedure to ascertain that they understood the technique. Besides, the parents were reminded to maintain their children on normal eating habits during the study.

The dietary record forms were collected every morning. The records were checked to clarify any unclear items or errors. Two home visits were arranged for each child. The first visit was on the first day of the study. This helped to resolve any problems encountered during the initial study period. The last visit took place on the last day of the study period at which the overall completed record was checked through.

The food items were coded and nutrient contents were analysed by the food composition table used in China (Institute of Health, Chinese Academy of Medical Sciences 1980).

In order to reveal the quality of dietary intakes in these children in the past five years, a questionnaire was designed to collect dietary information of the 16 children by interviewing the mothers. The sample questionnaire is shown in Appendix II.

6.2.4 Measurement of Bone Mineral Content

The technique and the procedures in measurement of bone mineral content in these 115 children were exactly the same as those performed in the children from Hong Kong.

Informed consents were obtained from parents to carry out bone mineral measurement by single photon absorptiometry in their children.

In order to make sure that the accuracy and precision of the instrument (in performing bone mineral measurement in China) could be comparable to those measured in Hong Kong. Multiple measurements (three to four scans each) with the same four chambered bone phantom were performed daily. Both the measurements of BMC and BW as measured by the instrument were highly correlated with the values in the bone phantom ($r = 0.999$). The coefficients of variation of multiple measurement of BMC and BW were 0.64 % to 2.94 % and 0 % to 0.62 % respectively. Therefore, it is confident that the accuracy and precision of the instrument in measuring BMC and BW in China are comparable to those measurements performed in Hong Kong.

6.2.5 Results

The results of dietary assessment of some nutrients of interest in 16 Jiangmen children is shown in Table 14. The dietary intakes of calcium, energy, protein, fat and carbohydrate were in general consistent with the findings of the 1982 National Dietary Survey in China (Ho 1988) and dietary survey in Beijing, 1989 (陈钧同寅 1989). The average calcium intake in Jiangmen children was less than 300 mg/day. Therefore, the mean calcium intake from these 16 children might be extrapolated to represent the average level of calcium intake in the whole group of children. When compared with the dietary intakes of Hong Kong children at age five (see Table 14), the mean calcium intake of Jiangmen children reached about half the amount (244 mg/day) taken by the Hong Kong Children, mean energy intake was about 11 % less than the intake of the Hong Kong children, and protein intake was about 27 g less than the intake of Hong Kong children. The intakes of fat and carbohydrate were similar in both groups.

From the dietary questionnaire, it was found that the dietary practice in this place is similar to many areas of Mainland China in that the babies were weaned off from milk around 12 months of age, milk was seldom consumed after weaning, and traditional rice based congee was commonly given to children at around four months of age. Minced meat, fish or egg and vegetables mixed with congee were gradually given to the babies until about one year of age when the babies could manage mini-adult diet.

Imported fortified cereals were expensive and therefore not popular among these children. Calcium and Vitamin D supplements have not been regularly used by the children.

At five years of age, most children were taking traditional Chinese diet. Milk was not commonly consumed. Occasionally they would drink condensed milk at home. In the kindergarten, milk was given to children two to three times a week, the milk powder was reconstituted more diluted than the full strength milk. Beans, nuts and dried fruit were not regularly consumed. Small fish, canned fish and dried shrimps with shell were taken occasionally. It appeared that these food items were not the major contribution of calcium in the diet. Cereals (mainly rice) and vegetables were two important sources of dietary calcium in these children.

The body size and bone width in these children have moderate correlations with BMC (see Table 15) and these findings were consistent with those in Hong Kong that body size and bone width are better predictors for BMC.

The results of body weight, height, BMC and BW of the Jiangmen children are shown in Table 16. The BMC of Jiangmen children was 0.043g/cm less ($P<0.01$), weight was 1.8kg lighter ($P<0.01$) and height was 4.5cm shorter ($P<0.01$) than Hong Kong children. It can be concluded the BMC of Jiangmen children is significantly lower than Hong Kong children.

What accounts for the difference in BMC in the two areas? Certainly it is not due to racial difference because both groups are originated from the same province of China. Is the difference in BMC due to calcium intake or body size? A stepwise multiple regression model was applied. After adjusting for body weight, height and bone width of these two groups of children, the effect of the mentioned variables only accounts for 25% of the difference in BMC. The remaining 75% difference in BMC cannot be explained by differences in calcium intake or body size in the two areas.

In order to test the assumption that the high BMC of Hong Kong children when compared with the Jiangmen sample was not attributable to high calcium intake at age five. The BMC of a sub-group (n = 37) of Hong Kong children with calcium intake below 344mg/day (the maximum calcium intake of Jiangmen children) was compared with 16 Jiangmen children. The sub-group of Hong Kong children has mean calcium intake of 264mg/day (SD = 52.09) and BMC of 0.310g/cm (SD = 0.042). The sub-group of Jiangmen children had mean calcium intake of 244mg/day (SD = 45.8) and mean BMC of 0.274g/cm (SD = 0.037). The difference of calcium intakes at age five of these two sub-groups is not significant (p = 0.1), while the BMC of the Jiangmen children is significantly lower (p = 0.003). Such difference also remained significant after adjusting for weight, height, bone width and sex. (p = 0.0034).

In other words, it can be concluded that the regional difference in BMC cannot be explained by calcium intake at age five. It is possible that the cumulative intake of calcium may be a better variable in explaining such a difference. However, as such dietary information was not available in the Jiangmen sample, such hypothesis has to remain untested.

Table 13

Current daily intakes of calcium, energy, protein, fat and carbohydrate of pre-school children (3 - 6 years) in China

	Nationwide Dietary Survey in China, 1982 (Ho ZC,1988)	Dietary Survey, Beijing, 1989 (陈钧同寅 1988)
Calcium (mg/day)	317.6	311.4
Energy (Kcal/day)	1292.0	1257.0
Protein (g/day)	35.2	36.3
Fat (g/day)	44.7	49.8
Carbohydrate (g/day)	183.3	167.7

Table 14

Comparsions of nutrients intakes of Chinese children at five from Hong Kong and Jiangmen

	Hong Kong		Jiangmen
	Girls (n=63)	Boys (n=70)	All (n=16)
	Mean (SD)	Mean (SD)	Mean (SD)
Energy (Kcal)	1325 (254)	1489 (316)	1245 (211)
Protein (g)	62.5 (16.3)	68.9 (17.3)	40.3 (8.2)
Fat (g)	39.9 (13.0)	42.9 (13.5)	42.7 (7.3)
Carbohydrate (g)	176 (33)	206 (46)	174 (32)
Calcium (mg)	525.3 (298.5)	536 (237.2)	244 (46)

Table 15

Correlation coefficients (r) of bone mineral content (BMC) with Bone Width (BW), weight and height of five years old Jiangmen Chinese children

	r	p
Boys (n = 63)		
BW (cm)	0.655	< 0.001
Weight (kg)	0.486	< 0.001
Height (cm)	0.477	< 0.001
Girls (n = 52)		
BW (cm)	0.583	< 0.001
Weight (kg)	0.581	< 0.001
Height (cm)	0.518	< 0.001

Table 16

Comparisons of mean weight, height, bone mineral content (BMC), bone mineral density (BMD), bone width (BW) and calcium intake of 128 Hong Kong Chinese children with 115 Jiangmen Chinese children

	Hong Kong Children	Jiangmen Children	
	Mean (SD)	Mean (SD)	p
Weight (kg)	17.4 (2.5)	15.6 (1.6)	< 0.01
Height (cm)	108.1 (4.2)	103.6 (4.0)	< 0.01
BMC (g/cm)	0.317 (0.042)	0.274 (0.037)	< 0.01
BMD (g/cm^2)	0.420 (0.040)	0.367 (0.038)	< 0.01
BW (cm)	0.756 (0.074)	0.750 (0.079)	NS
Calcium (g/day)	#546 (325)	*244 (46)	< 0.01

* No. of children = 16
\# No. of children = 133
NS denotes not significant

Chapter 7

Discussions

An assessment of dietary calcium intake and determination of the relation between calcium intake and bone mineral content in a group of Chinese pre-school children in Hong Kong have been carried out.

7.1 Reliability of Dietary Calcium Assessment

Every effort has been made to reduce bias in obtaining dietary information. The approach in asking parents in reporting habitual intake of food has greatly increased the accuracy of food recall. The use of selected food groups comprising different sources of food rich in calcium as a supplement to dietary history helped to reduce errors due to under reporting. For instance, food items like tofu and ice-cream might be forgotten to be reported by some parents during dietary taking but were eventually included during dietary assessment by food frequency method. Besides, a final cross-check with a 24 hours recall provided an additional validity of the dietary information given.

The parents involved in the co-hort study have been continuing in reporting dietary information of their children for the past four years. They are used to reporting this kind of dietary interview. The information provided are trustworthy.

The dietary assessment methodology had not been validated because the method used in this study was primarily designed to determine the habitual intakes over persisting long period of time. As mentioned earlier, Huenemann and Turner (1942) took six weeks using weighed intake method to validate dietary history method. The dietary history method was found to be highly correlated with the six-week weighed intake method for assessing calcium was good (r=0.97). However, it was not possible at this stage to carry out such a time consuming and labour intensive validity study.

Since milk occupied over 40% of the total calcium intake in these children, the brands of milk, volume and reconstitution have been recorded in details. Therefore, a large proportion of calcium intake should have been included during the dietary assessment. The food table used in the study for analysing calcium content in food is correlated with results obtained from chemical analysis in the validity study (r=0.8), which is reasonably accurate when compared with those quoted in the literature (Bingham 1987).

7.2 Reliability of Bone Mineral Measurement

As mentioned in Chapter 4, the single photon densitometer was highly accurate (r=0.999) and precise (<2% error) to measure BMC and BW when compared against the standard phantom. The reproducibility of the technique in measuring forearm BMC was very high: the coefficient of variation was less than 2.5% without subject repositioning, the intra-class correlation between measurements with subject repositioning was high ($r > 0.9$). These levels of precision were comparable to other studies quoted in the literature (Sorensen & Cameron 1967; Steichen et al. 1988). The accuracy and precision of the single photon densitometer were also tested when used in China. The instrument gave similar accuracy and precision in measuring bone mineral of children in China. Therefore, it is confident that the difference in BMC between two areas is not due to observational error.

7.3 Representative of Studied Children in Hong Kong

As mentioned earlier, the children in the study were recruited five years ago by means of stratified sampling according to the types of housing (Goldstein 1986). Fifty five percent of the study families were living in public housing as compared to 50% in Hong Kong population reference. Therefore, the families in the lower socio-economic groups have not been over represented in the study. Furthermore, judging from the fathers' occupations it also revealed that the different spectrums of socio-economic classes were represented in the studied group. Therefore, the sample should well represent the children population in Hong Kong.

7.4 Implications of the Study

7.4.1 Calcium Intake and Bone Mineral Content in Hong Kong Chinese Pre-school Children

The mean intake of calcium in Hong Kong Chinese children at five was 546 (SD=325) mg/day. One of the methods in interpreting the adequacy of a specific nutrient in a group of individuals is to compare the mean intake of the group with the RDA. The range of RDA for calcium in children varies from 400-1000mg/day (Nordin & Marshall 1988). Based on available research data in the literature, nutritionists in the world interpret the requirements of calcium for children in different ways. The RDA for calcium suggested by FAO/WHO (1962) and National Research Council (1989) in the U.S.A. are examples of different philosophies.

FAO/WHO (1962) considered that they could not define a precise minimum requirement for calcium in various age groups and they realised that calcium intakes in different parts of the world vary from 300 to over 1000mg/day, these people are able to adapt to their habitual levels of intake as a result of physiological adaptation in handling calcium. There has been no unequivocal evidence of calcium deficiency due to low intake. FAO/WHO (1962) recommends a safe practical allowance of 400-500mg/day calcium for children.

On the other hand, the RDA for calcium recommended by National Research Council (1989) in the U.S.A. advocates a generous allowance of intake to cover a wide margin of safety above the needs of most healthy population in that country. The estimation of requirements, as has been discussed in the introduction chapter, was extrapolated from balance studies in Caucasian adult populations whose calcium and protein intakes were relatively higher. Little consideration of nutritional adaptation was taken into account in setting up the RDA in children (800 mg/day). Furthermore, the National Research Council (1989) sets the RDA two standard deviations above the mean requirement with an intention to protect virtually the entire population. Therefore, it imposes an obligation to include a relatively generous margin of safety above the mean requirement. Consequently, failure to meet the RDA for calcium should not be considered as an evidence of inadequate intake. However, the further the actual intake deviates from the RDA, the greater the risk of inadequacy.

Therefore, it must be careful to make comparison with these RDA. The mean daily intake (546 mg/day) in Hong Kong Chinese children meets the recommended level suggested by FAO/WHO (1962). Since the U.S. RDA (800 mg/day) (National Research Council 1989) has been set at two standard deviations from the mean requirement, the mean requirement of the U.S. RDA may be inferred to around 500 - 600 mg/day (Nordin & Marshall 1988). In this sense, the mean intake of calcium in Hong Kong Chinese children is comparable with the mean requirement of the U.S. RDA.

The mean daily calcium intakes of these cohort children in the previous years are shown in Figures 9 and

10. It must be emphasised that 90% of the studied children have been taking milk regularly since birth. This levels of calcium intakes were attributable to regular use of milk. At five years of age, most of the children took in average a glass of milk daily. Although it is not comparable with the quantity of milk consumed in the milk drinking population. The inclusion of a glass of milk a day in a traditionally non-milk drinking population reflects a significant change in dietary habit. Given the fact that the mean intake of the whole group of children is comparable with the RDA and these cohort children are healthy and growing normally, the calcium intake of Hong Kong Chinese pre-school children should be adequate.

Studies in Hong Kong and Jiangmen consistently found that body weight, height and bone width are significantly correlated with bone mineral content. This implies that in determination of bone mass of children, the body size and bone width are important determinants to be considered. This findings are consistent with other studies in children (Mazess & Cameron 1971, Specker et al 1987). Such relations were also noted in two recent studies in adults : Kelly et al (1990) demonstrated that body weight, height and body mass index in adult men were significantly correlated with the axial and appendicular bones. Picard et al (1988) showed that body weight and height were significantly related to bone mineral content of premenopausal women.

The present study has shown that the variation in calcium intakes at five years cannot explain the difference in BMC of Hong Kong Chinese children even after adjusting for variables such as sex, weight, height and bone width. However, the cumulative calcium intakes over the first five years of life (in other words, the average daily calcium intake over the first five years period) is found to correlate with BMC. Moreover, the study indicates that calcium intake between 15 to 24 months of age is the most significant period in the first five years of life on predicting BMC at five years. Furthermore, it also confirms that the higher the calcium intake over the first five years, the higher the BMC. By grouping Hong Kong Chinese children into low, medium and high calcium intake groups in terms of average daily intake of calcium over the first five years of life the high calcium intake group and the medium calcium intake group are consistently found to have higher BMC than the lower intake group.

This observation is unique in a sense that the physiological process of bone mineralisation in childhood is a cumulative process. The bone mineral mass of the developing skeleton is proportional to the amount of calcium retained during growth. Therefore, the total bone mineral mass or the total skeletal calcium determined at any time during the years of skeletal growth is the resultant of bone mass accumulated over the past years of life.

Walker (1972) observed different population groups of low calcium intakes and bone dimensions. He concluded that low intake of calcium does not prejudice bone dimensions in different age groups. It is noteworthy to indicate that most of these studies reviewed by Walker were cross-sectional studies. In the present study, a similar conclusion might have arrived if the association between calcium intake and BMC were only related at the age of five in a cross-sectional manner. Hence, the present study suggests that the relation between calcium intake and bone mineral mass in growing children is more valid to be explained in longitudinal studies.

This is an important study in pre-school children relating long term calcium intake and bone mineral content. The study recorded with precise dietary calcium assessment. The findings from the present study demonstrates that long term calcium intake being significantly associated with BMC is consistent with those results in adult population. Halioua & Anderson (1989) studied the life time calcium intake of premenopausal women aged between 20 to 50 by recalling their calcium intake during their high school age, college age and adult life. The authors observed that life time calcium intake spreading over a long period of time was significantly correlated with BMC at the radial bone. When the life time calcium was divided into low (<500mg/d), medium (between 500 and 800 mg/d) and high (>800mg/d) groups, both the medium and high calcium intake groups were significantly correlated with greater bone mass at the radial sites. Kelly et al (1990) found that a higher calcium intake in adult men was associated with greater bone mass in the distal radius. Picard, Ste-Marie, Carrier, Chartrand, Lepage & D'Amour (1987) conducted a retrospective study on the effect of calcium intake between the age of 20 to 40 in 197 premenopausal women aged 40 to 50 at the time of the study. The study showed that the BMC of the lumbar spine and distal forearm was related to calcium intake in early adulthood. The BMC of the lumbar spine was significantly greater in the higher calcium intake group (>1,000mg/d) when compared with the medium intake group (between 500 and 1,000mg/d). These studies indicate the appropriateness of using long term calcium intake data to relate current bone mineral mass. In addition, calcium intake during early stage of life (i.e. adolescence and early 20s) was significantly associated with adult bone mass.

It has been mentioned in chapter 5 that calcium intake is proportional to the volume of milk taken in these children. Does it mean that children in Hong Kong should maintain regular milk drinking habit at least until age five? Although the results of the study indicate that higher BMC is associated with cumulative calcium intake in the past five years, the significant effect of having higher BMC in children at five on peak bone mass at their twenties or thirties has not been studies which warrants further investigation.

In a proportion of non milk drinking adult Hong Kong Chinese, cereals, green leafy vegetables, legumes and calcium precipitated tofu are major sources of calcium in these people (Pun et al. 1989). Bone, especially soft bone of fish and poultry as well as dried shrimps with shell are rich and unrecognised sources of calcium. Few studies have been done to determine phytate, oxalate and fibre in cereals, legumes and vegetables that are commonly consumed by the Chinese. And the calcium available for absorption from tofu, beans and soft bone is not fully understood. When calcium bioavailability in these food becomes fully understood these food may serve as milk alternatives in the Chinese diet. Meanwhile, that calcium from milk is readily absorbable (Nordin & Marshall 1988) renders milk an important source of calcium for children who are not milk intolerant.

Although the study indicates that cumulative calcium intake is an importance determinant on BMC, one would argue that the prediction of BMC by cumulative intake of calcium might be confound by some variables such as cumulative intakes of energy and protein as these variables are known to affect growth and bone development in children. By using multiple regression analysis, however, indicated that cumulative calcium intake remains an independent determinant on BMC even adjusted for cumulative intakes of protein and energy. Moreover, these two variables are not significantly correlated with BMC (partial $r=0.014$, $p=0.8867$ and partial $r=0.061$, $p=0.5368$ for cumulative intakes of protein and energy respectively). The results indicate that cumulative intakes of protein and energy in the first five years of life do not have any significant prediction of BMC in children at five years. Interestingly, Kelly et al (1990) also reached such a similar conclusion that bone mass in adult men was related to long established calcium intake habits but was independent from energy intake.

It may be argued that the low calcium intake group with low BMC might render poor bone mineralisation and reflects a lower body calcium store. It must be emphasized that the magnitude of BMC is only the bone mineral mass per centimeter of bone which is not an absolute measure of bone mineral mass though BMC at the distal radius is well correlated with total body calcium (Christiansen et al. 1975). BMC may be regarded in much the same way as blood pressure, it is an actual physiological measurement. It requires a normal reference from the local population to compare with the BMC obtained in the study. Yet, this normal reference is not yet available in Hong Kong. There are very few normal references of BMC in children as little as age five in the literature. It is not appropriate to compare with the American normal reference (Mazess & Cameron, 1974) because the lowest age group in their study children is six and the measurement site is at the mid-shaft of the radius instead of the distal one-third site.

There were 21 cohort children with average daily calcium intakes over the past five years period below 400mg/day. When the individual records of health and dietary intake were retrieved, those children with calcium intake below 400mg/day have been healthy since birth, none of them have suffered from any major illness. All of them have been taking milk up to two years. There were 12 children abstained from milk drinking from 24 months to 60 months : three children at around 24 months, three children at about 36 months, four children at 48 months and two children at 60 months. The other nine children were still taking milk; however, the consumption was irregular or the volume of milk consumed was low.

Furthermore, the cohort children at different levels of calcium intakes have been healthy, well nourished and growing normally in their early life (Leung & Lui 1989; Leung et al. 1989). It does not appear that low BMC in these Hong Kong Chinese children is deleterious to the current state of health in spite of the fact that we cannot at this stage define the normal range of BMC in Chinese children.

Speaker et al. (1987) found that there was a significant sex difference in BMC of children from five years onwards but this difference was not seen in the Hong Kong Chinese children. However, there is a sex difference in bone width which might reflect a difference of bone size in boys and girls.

7.4.2 Calcium Intake and Bone Mineral Content of Jiangmen Pre-school Children

The dietary calcium intakes of children in Jiangmen was in general lower than the counterparts in Hong Kong. Mean calcium intake in these children was half of the amount taken by the Hong Kong Chinese children. The low calcium intake of Jiangmen Chinese children is attributable to low milk intake which is consistent with the result of a nation wide dietary survey in China in 1982 (Ho 1988). The body size of the children in Jiangmen is also significantly lower than the Hong Kong Chinese children. This may be partially attributable to lower dietary intakes of energy and protein in Jiangmen children.

It is noteworthy that the difference in BMC of children in these two regions cannot be fully explained by difference in calcium intakes even when the major determinants on BMC (i.e. weight, height and bone width) are adjusted. Furthermore, when a sub-group of Hong Kong Chinese children (calcium intake < 344mg/day) whose calcium intakes are comparable to those of the 16 Jiangmen children after adjusting for body size, bone width and sex, a significant difference in BMC still exists between the two groups of children. Certainly, if the cumulative intake of calcium in these children were available, it would have provided a better explanation for the difference in BMC in the two regions.

It has shown that using multiple regression model, variables of average calcium intake in the first five years, body size, bone width and sex could only explain for 55% of the variance of BMC in the Hong Kong children.

Furthermore, in comparing two sub-groups of children with similar range of calcium intake from Hong Kong and Jiangmen, even after correcting for body size and sex, the difference in BMC still remains. A possible significant factor that has not been tested in determining BMC in children is the level of physical activity in these children. Mechanic stress due to physical exercise seems to enhance bone formation and cortical bone mass (Dequeker 1988). Halioua and Anderson (1989) demonstrated that a higher life time physical activity (i.e. more than 45 minutes of moderate to strenuous activity, four times a week) was significantly related to bone mass in premenopausal women. The authors indicated that adequate calcium intake and physical activity are important factors in enhancing peak adult bone mass.

In Jiangmen, the kindergarten requires the children to sleep in bed for three hours in the afternoon. Whereas in Hong Kong most of the studied children do not sleep for such long hours even if they attend whole day kindergarten. In addition, it also observed that there is not enough space in the kindergarden for about 700 children to play and do physical exercise. As the children attend the kindergarten from 7:00pm to 4:00pm, five and a half days a week, the relation between the effect of being less active and BMC requires further study.

Most of these children in Jiangmen weaned off from milk around 12 months, since then little milk have been consumed. Whereas the counterparts in Hong Kong have been taking milk since birth. It has been mentioned that repeated dietary studies in China reported that calcium intake of Chinese pre-school children are about 300mg/day. It may imply that a habitual low intake of calcium in growing children over a persistent period of time predispose to low BMC. If cumulative intake of calcium in these children were available, it might have supported the conclusion that there is a significant association between cumulative intake of calcium from birth to five years and BMC as found in Hong Kong children.

Children from Hong Kong and Mainland China with low intakes of calcium are observed to have lower BMC. Would it be possible that growing children may not be able to fully adapt to a persistent low calcium intake ? Or there may be a threshold level of calcium intake below which the body may not be able to adapt and the bone will suffer from under-mineralization. Malm (1958) found that not all adult men could fully adapt to low calcium intakes, some of them failed to adapt and suffered from chronic negative calcium balance. It is not known whether this failure in adaptation might happen in children and would prejudice the quality and quantity of bone. One of the possible outcomes in children may be under-mineralization of bone (Fraser 1988b). Studies in South Africa reported that young children with chronically low calcium intakes developed rickets which was not due to vitamin D deficiency (Pettifor et al.1978; Eyberg et al. 1986). It may require more studies to investigate this phenomenon.

7.5 The Use of Bone Mineral Density (BMD) As An Parameter in Representing Bone Mineral Mass

Although the study findings indicate that the current and cumulative intakes of calcium are significantly associated with BMD. The BMD (i.e. the ratio of BMC to BW) is an unproved conceptual parameter and the use of BMC/BW ratio alone does not provide any specific advantage and theoretically could mask the important information of bone mass and bone size (Mimouni & Tsang 1988). It is not certain whether cortical bone in growing children grow proportionally to bone width because the medullary cavity is also changing in growing children. The diameters of both endosteal surface and the periosteal surfaces are expanding, it is not known whether BMC may increase at the same rate as that of the periosteal bone width. Therefore, the actual BMD is not necessary falling in children with wider bone width (Filer 1988).

In addition, the use of the ratio BMC/BW to correlate with other variables would degrade the whole statistical analysis because it is dealing with multiple comparisons and the Bonferroni principle needs to be applied. However, Bone mineral content (BMC), an independent variable and a covariance matrix can be used to express bone mass quantitatively such that the statistical correlation would be adjusted for bone width and weight (Filer 1988). Therefore, the statistical analysis using BMD as a dependent variable in the present study only serves for reference purpose. The appropriateness of using BMD to correlate the relations between bone mass and other variables requires further investigation.

7.6 A Need for Further Studies

In order to confirm whether calcium intake has a significant effect on BMC, a controlled trial of supplementing children with calcium for a period of time and investigate if there is any significant improvement in the BMC. The study groups will be ideally children from Jiangmen and children from Hong Kong with low calcium intake over the first five years of life and low BMC. The control group can be Hong Kong Chinese children who consume milk regularly.

Besides, it requires follow-up study to confirm the beneficial effect of higher BMC in children at five that may influence peak bone mass in early adulthood.

Furthermore, it will be important to study the calcium requirements of children subject to varying levels of calcium intakes in order to see whether children are able to adapt to low calcium diet. The interrelationship between calcium and dietary components such as vitamin D, protein, salt, phosphorus, carbohydrates, phytate, oxalate, etc., are important factors in influencing calcium bioavailability. Hence, these dietary factors deserve attention when studying calcium requirements in children.

7.7 Conclusions

The dietary calcium intake of pre-school Chinese children in Hong Kong is comparable with the RDA (FAO/WHO 1962; National Research Council 1989). Most of the children have been drinking milk since birth. Milk and milk products contribute about 43% of total calcium in the diet. This reflects a substantial change in dietary habit in the younger Chinese population in Hong Kong. These children are healthy and growing normally on the current level of calcium intake (546mg/day). Therefore, it suggests that the calcium intake of Hong Kong Chinese pre-school children is adequate.

A significant relationship was found between cumulative calcium intake over the first five years of life and BMC at five years, the higher the calcium intake over the first five years, the higher the BMC. Moreover, calcium intake between 15 to 24 months of age appears to be the most significant determinant on BMC at age five. These observations suggest that calcium intake on a long term basis has an significant effect on BMC in growing children. Attempt to relate BMC and calcium intake by cross-sectional study does not seem to be a valid method to reveal the actual relation between calcium intake and BMC in children.

It was also found that the difference in BMC among Hong Kong Chinese children, and between the counterparts from mainland China cannot be fully explained by difference in calcium intakes. It is speculated that cumulative calcium intake and the level of physical activity may be some of the important determinants in explaining such difference in BMC.

Calcium intake between 15 to 24 months has been found a crucial stage of life on predicting BMC in children at five years. This finding may suggest that for the benefit of higher bone mass, Children from Hong Kong should maintain milk drinking habit until at least two years of age. And children in Mainland China should not wean off from milk by one year of age, they should continue the habit of milk consumption until at least two years of age.

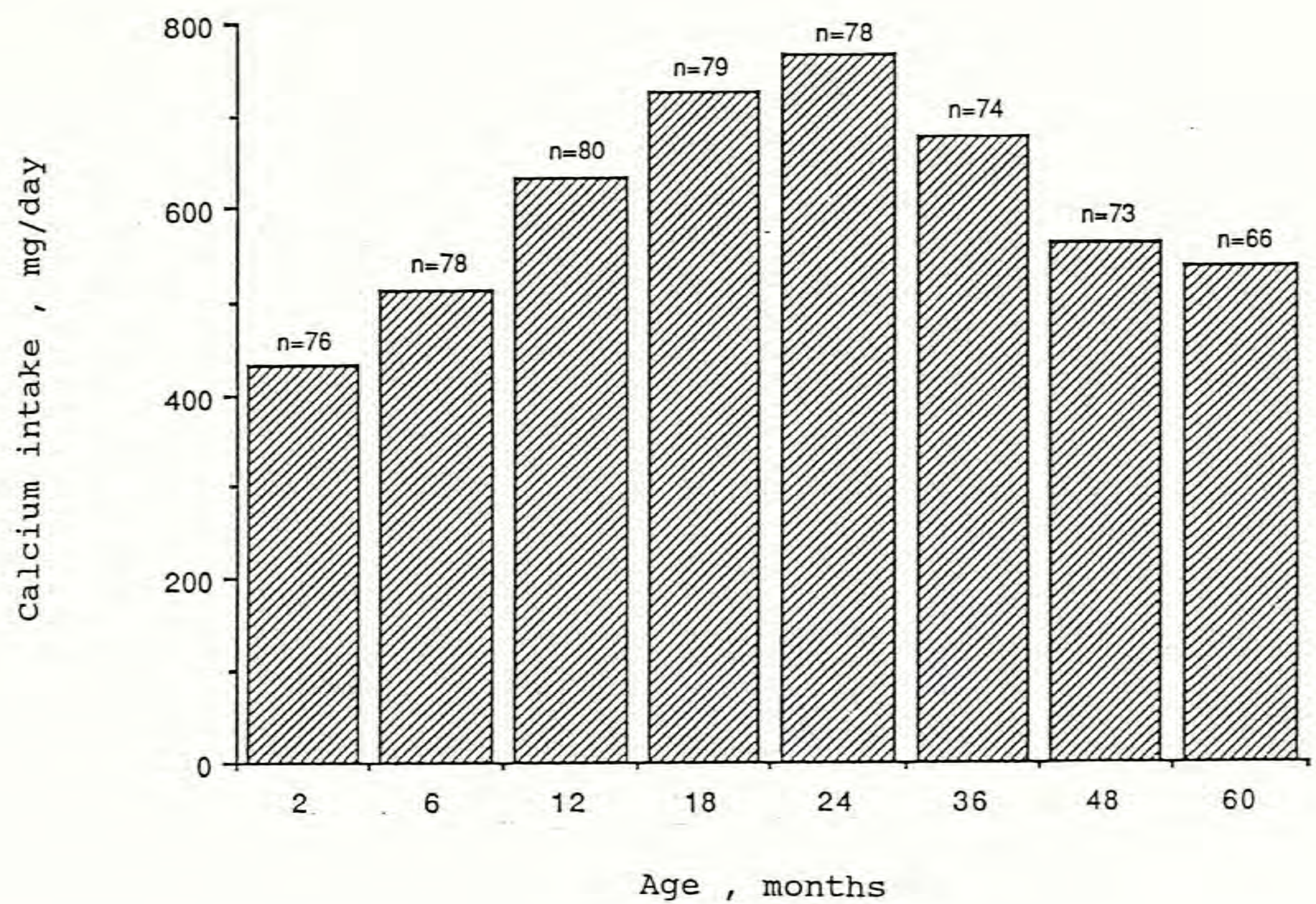

Figure 9 Mean daily calcium intakes in Hong Kong Chinese boys from birth to five years of age

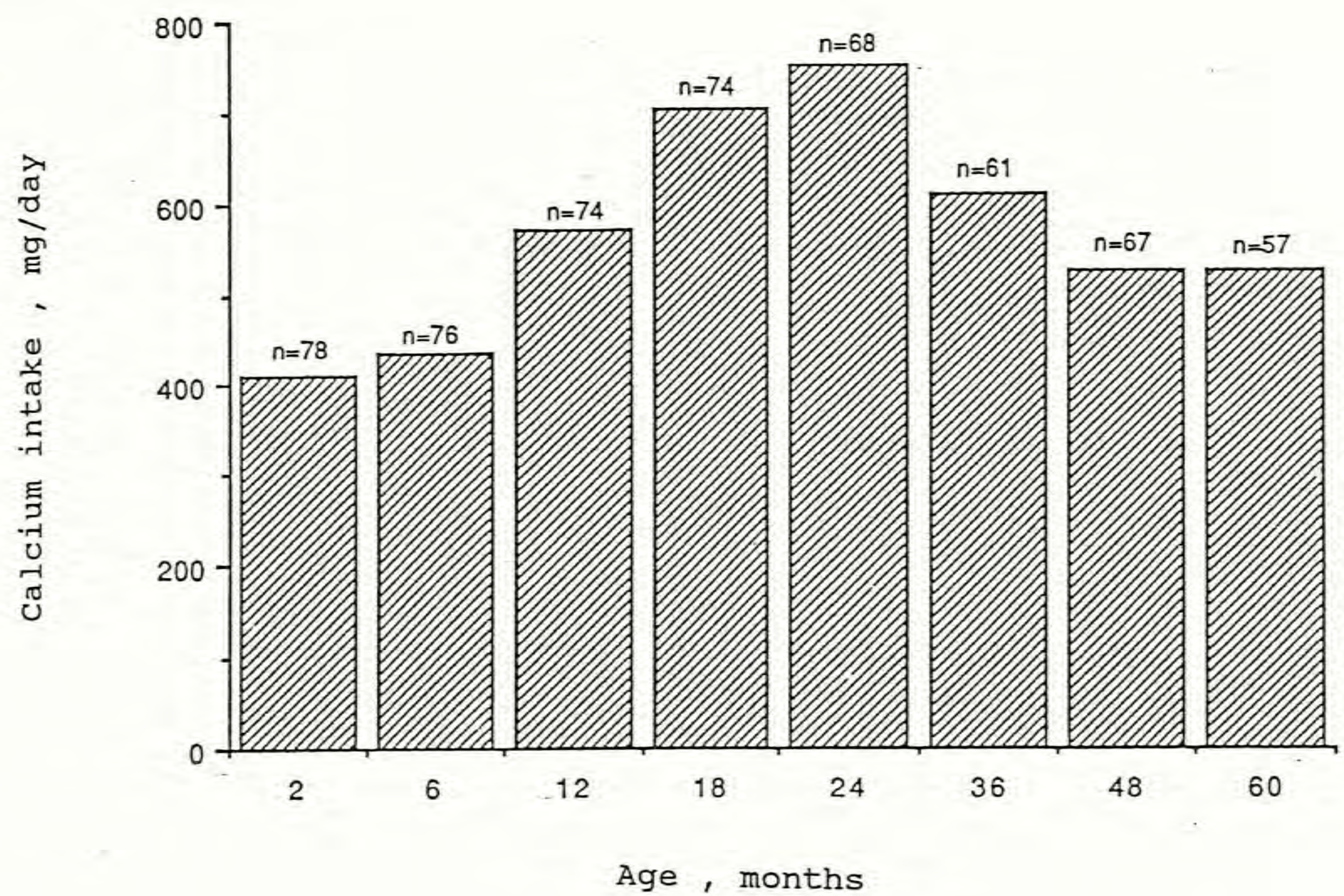

Figure 10 Mean daily calcium intakes of Hong Kong Chinese girls from birth to five years of age

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Appendix I

Dietary Record Form Used in Hong Kong Study

Name :______________________

Ref.No.:____________________ Date :_______________

(I) **Dietary History Record** (Please specify the amount and frequency of food taken in the last two month)

Breakfast

Lunch

Dinner

Fruit and fruit juice

Snack Food

Fast Food

Miscellaneous. eg. barley water, chrysanthemum tea

(II) **Food Frequency Record**

(A) Milk and Milk products

(1) Does the child consume formula milk ?
Yes ( ) No ( )

If yes, what type ? ____________________

How much ? ______________________________

How often ? _____________________________

How is the formula prepared (proportion of milk powder to water) ?

----

(2) Does the child drink milk ?

Yes ( )   No ( )

If yes, what kind ? ____________________

How much ? ____________________

How often ? ____________________

(3) Does the child take dairy products , eg. cheese, yoghurt or ice-cream, etc. ? (Please specify the type, amount and frequency)

(B) Bread & Cereals

(C) Vegetables

(D) Beans & Bean Products

(E) Nuts and Seeds

(F) Dried Fruits

(G) Small fish eaten with bone, Tinned fish eaten with bone, dried shrimps with shell, etc.

(H) Does the child take calcium supplements ?
Yes ( ) No ( )

If yes, What type ? ____________________________

The dosage ____________________________________

How often ? ___________________________________

(III) **24-hour Recall**

(Please list all food or drinks on a typical day)

Meal 1 Time : ________ Place : ________

Meal 2 Time : ________ Place : ________

Meal 3 Time : ________ Place : ________

Meal 4 Time : ________ Place : ________

Meal 5 Time : ________ Place : ________

Meal 6 Time : ________ Place : ________

Meal 7 Time : ________ Place : ________

Meal 8 Time : ________ Place : ________

Meal 9 Time : ________ Place : ________

Appendix II

Dietary Questionnaire Used in Jiangmen Study

ID : ____________________ Date : ________________

Name : ____________________

Sex : ____________________

D.O.B. : ____________________

Address:

____

(I) Dietary History Before Five Years

1. Was the child breast-fed ? Yes [ ] No [ ]

   If yes, it lasted until __________ months.

2. Did the child receive infant formula ?
   Yes [ ] No [ ]

   If yes, Started from ______ month to ______ month.

   Which kind ? ____

   How much ? ____

   How often ? ____

   How was the formula prepared (especially dilution) ?

   ____

3. Did the child drink milk, e.g. fresh milk, powdered milk, or condensed milk ,etc. ?
   Yes [ ] No [ ]

   If yes, what kind ? ___________________________________

   ____

When did the child start and when did he/she terminate it.

____

How much ? ______________________________________

____

How often ? ______________________________________

4. Did the child take any baby rice cereals ?
Yes [ ] No [ ]

If yes, What kind ? a) home-made ?
Yes [ ] No [ ]

b) Commercial product ?
Yes [ ] No [ ]

If yes, what type ?

____

____

Fortified ?
Yes [ ] No [ ]

Started from _______ month until _______ month

How much ? ______________________________________

How often ? ______________________________________

5. Did the child take rice congee ?
Yes [ ] No [ ]

a) If yes, i) From ______ month until ______ month

ii) How much ? ___________________________

b) Was meat, fish or egg, etc., added to cook with congee ?
Yes [ ] No [ ]

If yes, How much ? ______________________________

____

c) Did the child eat the solid in the congee ?
Yes [ ] No [ ]

If yes, How much ? ______________________________

____

6. Did the child take moisted rice ?
Yes [ ] No [ ]

a) If yes, a) Started from _______ month until _______ month

b) How much ? ______________________________

____

c) Was meat, fish or egg, etc. added to the moist rice ?
Yes [ ] No [ ]

If yes, How much ? ______________________________

____

d) Did the child take the solid in the moisted rice ?
Yes [ ] No [ ]

If yes, How much ? ______________________________

____

7. When did the child start to take adult's meal ?

From ________ months

a) How often ? ______________________________

b) How much rice was taken at one meal ?

____

c) What kinds of dishes did the child usually take ?

____

____

____

d) How much ? ____________________________________

____

____

8. Did the child take any calcium and vitamin D supplements ?
Yes [ ] No [ ]

If yes, What kind ? ________________________________

How much ? ________________________________

How often ? ________________________________

(II) Food frequency record of calcium intake at five years of age

(A) Milk and milk products (including ice-cream)

____

____

____

(B) Cereals based products (including bread, biscuits)

____

____

____

(C) Bean and bean products (including lotus seed bun or red bean soup, etc.)

____

____

____

(D) Dried fruits (e.g. raisin, dates or prunes, etc.)

____

____

____

(E) Nuts

____

____

____

(F) Fruits and fruit Juice

____

____

____

(g) Small fish (eaten with bone), canned fish (eaten with soft bone), dried shrimps with shell)

____

____

____

Appendix III

Validity of the Compiled Food Composition Table

1.1 Introduction

To determine the accuracy of the compiled food table in analysing calcium content in food, a validity test was performed to compare calcium content in food obtained from the food table and the results obtained from chemical analysis.

Ordinary Chinese diet was collected over a seven-day period. Individual food items were weighed and the calcium content was calculated from the food table. The food was then treated by dry ashing and the calcium content was determined by atomic absorption spectrophotometry. The results obtained were compared with those calculated from the food table.

1.2 Materials and Methods

1.2.1 Food collection

Food was collected from a paediatric ward in Prince of Wales Hospital, Shatin, over a seven-day period. The diet collected was ordinary Chinese meal. The food service was provided by the hospital catering department. During each main meal at the food collection period, the ward attendant set aside a tray of food typically distributed to any one child of five years old in ward. The breakfast was usually a bowl of meat or egg congee; the mid morning snack contained fresh milk or chocolate milk and bread with jam; the types of food for lunch and dinner were mainly rice, different varieties of meat, fish, egg, vegetables, bean and bean products and fruits.

1.2.2 Food Pre-treatment

Bone from food like pork chop, fish and chicken was removed, fruit was skinned and the seed contained was removed. Therefore, only the edible portion of food was weighed and analysed chemically. This was important because nutrient data recorded in food table is mostly derived from edible portion of food.

1.2.3 Food Weighing and Determination of Calcium Content with the Compiled Food Table

A pre-weighed container of the blender (Philips, model : HR 1376) was placed on top of a electronic scale (Mettler, model: PE 2200) which was accurate to two decimal places. Each food item collected on the same day was weighed and recorded, then each food item was coded according to the coding system of the compiled food table. The food table had been computerized for speeding up the procedure of nutrient analysis. Therefore,the analysis of food calcium content was run by a IBM compatible personal computer.

1.2.4 Chemical Analysis

Modified Nordin (1976) method for food calcium analysis by dried ashing and atomic absorption spectrophotometry was used in this study. After weighing of food , 100 ml of deionised distilled water was added and the mixture was homogenized for ten minutes. About 100 g of the homogenate was stored in the freezer under -4 ^{o}C. for batch analysis.

All the samples were done in duplicate. 7 g of homogenate was added into a 15 ml silica crucible and the crucibles were placed in a sand bath at 100^{o}C for five hours until the samples were dry, the samples were then heated in a muffle furance (Sybron Thermolyne 2000 Furance) at 550^{o}C for 18 hours. After cooling, 10 ml of 5 molar HCl was added to dissolve the ash. The mixture with trace of carbon particles was centrifuged at 3,000 revolutions per minute for 15 minutes. 8 ml of supernatant was transferred to 100 ml volumetric flasks and then diluted to 100 ml with deionised distilled water. The aliquots were used for estimation of calcium concentration by atomic absorption.

1.2.4.1 Atomic Absorption

4 ml of 0.1 % lanthanium chloride solution (a chelating agent for phosphate) was added to 200 microlitre of samples; the working standards (calcium nitrate diluted to 4 mM, 2 mM and 1 mM); reference serum (Seronorm, Nycomed, Norway, for quality control) respectively and mixed well. Atomic absorption spectrophotometer (Varian, model: AA 1475) was used with the following parameter setting :-

Wavelength	:	422.7 nm
Slit width	:	0.5 nm,
Lamp current	:	5 mA
Mode	:	Double beam
Fuel	:	Acetylene - 6 psi
	:	air - 17 psi

1.2.5 Evaluation of the Method of chemical analysis

The accuracy and precision of the chemical method in analysing calcium content in food had been studied with a batch of dried cow's milk powder with known content of calcium (793 mg/100 g). Ten samples of the milk powder were weighed and treated as mentioned above. The analytical mean was 678.15 mg /100 g (SD = 22.80). The analytical mean concentration of calcium was 14.5% less than the true value and the coefficient of variation was found to be 3.2 %. Therefore, the analytical procedures employed in this chemical method would be reasonably accurate and reliable to determine calcium content in food.